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Improving Synchronization and Functional Connectivity in Autism Spectrum Disorders Through Plasticity-Induced Rehabilitation

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14. ABSTRACT The rationale for using neurofeedback to affect changes in children on the autism spectrum is rooted in several assumptions. First, regions comprising the human mirror neuron system or MNS exhibit abnormal connections in ASD children. Second, the 8-13 Hz mu rhythm oscillations over sensorimotor cortex are functionally linked to the MNS network. Third, modifying these oscillation dynamics via neurofeedback training induces neural plasticity. Finally, normalization of abnormal connectivity is reflected in positive behavioral, cognitive, and electrophysiological changes. Results from this grant show behavioral improvements following training in all the subcategories of the ATEC, a validated measure of the efficacy of an intervention. Similarly, we report improvement in the three subscales in adaptive measures, as measured by the Vineland Adaptive Behavioral Scale. Finally, social interactions during daily living were assessed with the Social Responsiveness Scale and improvements noted with training. We also report functional neuroanatomical changes following neurofeedback training. Blood oxygenation level-dependent (BOLD) activation results indicate that both TD and ASD groups have activity in IFG, IPL, and STS areas during imitation and in the observation of hand movement, but these activations are significantly greater for the TD group. Furthermore, our results are consistent with anatomical findings that show regions in the ASD brain exhibiting overconnectivity and underconnectivity compared to the TD brain. Finally, we show that NFT produces increases in MNS activity and reductions in abnormal functional connectivities between MNS areas compared to the TD brain.

15. SUBJECT TERMS
Quantitative electroencephalography (QEEG), neurofeedback, autism spectrum disorders

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Improving Synchronization and Functional Connectivity in Autism Spectrum Disorders Through Plasticity-Induced Rehabilitation

FINAL TECHNICAL PROGRESS REPORT

1 August 2010 – 31 July 2013 (with NCE)

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INTRODUCTION

Autism is a highly varied developmental disorder typically characterized by deficits in reciprocal social interaction, difficulties with verbal and nonverbal communication, and restricted interests and repetitive behaviors. Although a wide range of behavioral, pharmacological, and alternative medicine strategies have been reported to ameliorate specific symptoms for some individuals, there is at present no cure for the condition. Nonetheless, among the many observations about aspects of the development, anatomy, and functionality of the autistic brain, it is widely agreed that it is characterized by widespread aberrant connectivity. Such disordered connectivity, be it increased, decreased, or otherwise compromised, may complicate healthy synchronization and communication among and within different neural circuits, thereby producing abnormal processing of sensory inputs necessary for normal social life. It is generally accepted that the innate properties of brain electrical activity produce pacemaker elements and linked networks that oscillate synchronously or asynchronously, likely reflecting a type of functional connectivity. Using phase coherence in multiple frequency EEG bands as a measure of functional connectivity, studies have shown evidence for both global hypoconnectivity and local hyperconnectivity in individuals with Autism Spectrum Disorder (ASD). However, the nature of the brain's experience-dependent structural plasticity suggests that these abnormal patterns may be reversed with the proper type of treatment. Indeed, plasticity induced rehabilitation also known as neurofeedback training (NFT), is an intervention based on operant conditioning that results in self-regulation of brain electrical oscillations, and which has shown promise in addressing marked abnormalities in functional and structural connectivity. It is hypothesized that NFT produces positive behavioral changes in ASD children by normalizing the aberrant connections within and between neural circuits. NFT exploits the brain's plasticity to normalize aberrant connectivity patterns apparent in the autistic brain. By grounding this training in known anatomical (e.g., mirror neuron system) and functional markers (e.g., mu rhythms) of autism, NFT holds promise to support current treatments for this complex disorder. The proposed hypothesis specifically states that neurofeedback-induced alpha mu (8–12 Hz) rhythm suppression or desynchronization, a marker of cortical activation, should induce neuroplastic changes and lead to normalization in relevant mirroring networks that have been associated with higher-order social cognition. The project funded by CDMRP investigated the functional correlates of ASD with the goal of developing strategies to reduce cognitive, behavioral, and neurofunctional deficits. The primary goal was to test a model of the neural basis for changes in ASD induced by QEEG-guided neurofeedback training (the mirror neuron hypothesis of autism). We proposed that this intervention would help characterize the effects of altering cortical dynamics via operant conditioning of the EEG mu rhythm (8-13 Hz oscillation over sensorimotor cortex) on the amelioration of ASD symptoms and its impact on matched, typically developing children. It would also help characterize the specific cognitive, behavioral, electrophysiological, and functional brain changes that occur with such training. The positive outcomes provided evidence for a link between "mirroring" activity in the human brain, EEG mu rhythms that reflect large-scale processing, and behaviors that comprise the core deficits in ASD. Furthermore, the project helped to identify behavioral phenotypes that may contribute to diagnosis of the disorder and help predict successful treatment outcomes.

BODY:

1. IRB review of protocol changes: Prepare and submit changes to the currently approved protocol

Preparation and submission of the paperwork to get the protocol approved began as soon as notice of the award was received. We started IRB protocol approval process at UCSD in January 2010 and received approval four months later in May 2010. We then submitted to CDMRP, but a number of issues arose that led to various delays in the start of the project. We were delayed for approximately two months, until August 1, 2010, primarily due to missing or delayed documentation (e.g., Facility Safety Plans for both UCSD and SDSU), as well as problems with the protocol approval process. We had requested money for neuroimaging scans on the main grant since costs to Dr. Pineda, as PI and UCSD faculty, would be slightly less at the UCSD Keck Neuroimaging Center. Unfortunately, a series of local misunderstandings led to requesting that this money, post-award, be directly allocated to the subcontractor. Because the request was approved, we were then in the unfortunate position of paying a higher rate for use of the scanner. We set about reversing this but it led to a couple more months of delay. Since PIRT or neurofeedback training is to be guided by a quantitative analysis of the EEG, it was necessary to set up a process for recording, analyzing, and implementing training protocols based on the outcome of the QEEG. We decided it was best to send the EEG data to an expert in the field for that type of analysis. Unfortunately, we did not initially budget sufficient funds for this aspect of the proposal. It took several months to negotiate a deal on the most cost-effective method to do this but our lack of funds led to our requests given very low priority. We eventually received a UCSD Senate grant to help cover these costs. Some delays were also due to technical problems with the bioamplifiers and impedance sensors that are part of the Thought Technology neurofeedback system. One particular early concern was a breakdown in the training of the RAs, coupled with the delicate nature of some of the hardware, which contributed to this problem. Perhaps the most serious delay occurred from another misunderstanding. Thinking that because we had local approval from the UCSD IRB for all aspects of the project we could start the recruitment process before the final approval of the protocol had been received from CDMRP. On March 28, 2011 we were notified of our noncompliance. According to the Provision 4 in the Assistance Agreement it is prohibited to enroll human subjects without the HRPO approval. A stop payment was placed on funding until such approval occurred. Another major issue developed in that in order to increase participant accrual we had established a partnership with a center in the Los Angeles area where we hoped to replicate the work being conducted at UCSD in San Diego. However, we first needed to modify the university's FWA to include this new research center in order for research to officially begin there. After many rounds we finally got local approval for the change in the FWA. Project received initial IRB approval on Aug 1, 2010.

2. Set up and test EEG protocols at study sites: Set up and test hardware and software for neurofeedback training at UCSD and protocols for neuroimaging at SDSU

This project was initially to take place at two study sites: The University of California, San Diego (UCSD) and San Diego State University (SDSU). However, in order to recruit additional subjects we established a third center at the Speech and Language Development Center (SLDC), a non-profit school in LA County dedicated to the education of neurologically handicapped children. The PI, Dr. Jaime A. Pineda, is faculty in the Department of Cognitive Sciences at UCSD and was responsible for all components of the project related to quantitative electroencephalogram (QEEG)-guided neurofeedback training, including recruitment of autism spectrum disorder (ASD) and typically developing (TD) participants, pre- and post-

training assessment, protocol preparation and testing, rehabilitation training, data analysis, and preparation for publication. The Co-Investigator, Dr. Ralph-Axel Mueller is faculty in the Department of Psychology at SDSU and was responsible for all components of the project related to neuroimaging, including preparation of functional magnetic resonance imaging (fMRI) designs and data acquisition protocols, contact and scheduling of participants for imaging sessions, mock scanning sessions (where appropriate), acquisition of structural and functional magnetic resonance (MR) images, preprocessing of MR imaging data, statistical analyses of functional MRI data (whole brain and region-of-interest analyses), and preparation for publication.

EEG protocols, including hardware and software for neurofeedback training, were successfully installed and tested at the Cognitive Neuroscience Laboratory at UCSD and SLDC, as well as testing protocols for neuroimaging at SDSU/UCSD. These continued to be active and generally problem-free through the length of the study. SLDC Research Assistants who administered and scored outcome measures, electrophysiological tests and neurofeedback training were trained. At the beginning of the project we had identified a School Psychologist, Sally Miller, to help verify the diagnosis of autism (ADI, ADOS) for each high functioning autistic child recruited to the study at SLDC. Sally had received initial trained by Dr. Alan Lincoln (our consultant) and subsequently was certified by *WESTERN PSYCHOLOGICAL SERVICES (WPS),* which is approved by the American Psychological Association (APA) to sponsor continuing education for psychologists. Ms Miller was also to administer the IQ test (Wechsler Abbreviated Scale of Intelligence or WASI) while also training SLDC personnel to administer these assessments themselves. Unfortunately Ms Miller moved from the area and was not able to continue this work. Fortunately, Dr. Lincoln arranged for the Los Angeles CARES office and for some in the San Diego office to provide the testing for the SLDC population.

3. Training of research assistants: Begin training graduate student at UCSD and postdoc at SDSU
We established protocols to train 6-8 RAs in the Cognitive Science program each quarter during the academic school year to provide neurofeedback training to our participants. The RAs were trained to administer and score the behavioral, cognitive, and electrophysiological outcome measures and administer neurofeedback training. We also identified two individuals dedicated specifically for scheduling the training times with parents and participants and making sure that at least two trained RAs were available during the sessions

4. Training of students on PIRT: Graduate student will be responsible for training a set of undergraduate students who will help with the day-to-day training of participants
Training students involved one-hour introduction to the program and the specifics of neurofeedback. Students attended 3-5 training sessions in which they first observed and then practiced the methodology on each other. This was followed by participation in actual training sessions with study participants. Student RAs weree assigned to a senior trainer and over the course of 3-5 sessions learned to conduct the protocol on their own. This worked quite well and provided continuity in the training of participants. Currently there are 6-8 RAs who have received the training and are conducting actual neurofeedback sessions. Some were also trained to administer and score the behavioral, cognitive, and electrophysiological outcome measures in addition to administering neurofeedback training. Graduate

student has been trained by the postdoc at SDSU on the administration and analysis of the neuroimaging protocols.

5. Advertise and recruit participants: Produce and distribute ads and flyers, as well as talk to various autism groups in San Diego

Recruitment efforts began in August 2010. There were three parts to this effort: advertise and recruit, select participants, administer pre-training assessments. Advertising and recruitment of participants involved producing and distributing ads and flyers, talking to various autism groups in San Diego, and contacting parents in our database who had previously participated in other studies at the university. We also advertised on the Autism Speaks website and other such websites. Finally, we contacted both our consultant, Dr. Alan Lincoln, who is Director of the San Diego Center for Autism Research, Evaluation and Service (CARES) and our collaborator, Dr. Ralph Axel-Mueller at SDSU, for referrals to our project. These efforts were carried out periodically to identify groups of children with ASD who met the criteria for participation and whose parents showed a willingness to be in the study. We also identified typically developing (TD) children for the control group.

Selection of participants required the verification of diagnosis, conducted by Dr. Lincoln at the CARES center in San Diego, and the administration of the Autism Diagnostic Observation Schedule or ADOS, Autism Diagnostic Interview or ADI, and Wechsler Abbreviated Scale of Intelligence or WASI. ASD participants were evaluated to determine if they met inclusion criteria for high functioning autism (IQ>80). Once identified, participants were scheduled for cognitive, behavioral and electrophysiological assessments at UCSD. These included evaluation of the Mu Suppression Index (MSI), quantitative EEG (QEEG), Emotion Discrimination, Autism Treatment Evaluation Checklist (ATEC), additional paper and pencil tests. Once those were completed participants were scheduled for a neuroimaging scan at the Center for Functional MRI, or W.M. Keck Building at UCSD. All participants, both TD and ASD, were evaluated in the same way.

Unfortunately problems developed from the outset in our recruitment efforts. Parents and children found it much more difficult to participate during the school year than during the summer months and thus our accrual of subjects slowed down considerably after September. Therefore, as noted above, in order to increase participant accrual we formed a partnership with SLDC located in Buena Park, California (2 hrs north of the UCSD campus) to essentially duplicate our efforts there. SLDC offered a readily available pool of children on the spectrum, who attended the school, as well as TD children. However, because of numerous problems, accrual at SLDC did not meet expectations. At the end of the study, we had recruited a total of 36 ASD and 15 TD participants to the study. Of that number a total of 30 participants (18 ASD and 12 TD) met the criteria for inclusion[1] and were successfully scanned in the pre-training phase. Of those, 25 participants (16 ASD and 9 TD) completed the post-training scans. Several ASD as well as TD participants dropped out without completing training or showed unusable data and could not be included in the data analyses.

[1] We initially recruited siblings of ASD participants as controls but learned that this was not appropriate so some presumed TDs had to be dropped.

6. Selection of participants (Clinical assessments: ADOS, ADI, WASI): Potential participants are evaluated by consultant to determine they meet inclusion criteria.

Dr. Alan Lincoln, Director of CARES in San Diego and project consultant, along with his staff were directly responsible for the clinical assessments, the scoring and interpretation of results. We set up an efficient system to schedule participants and test them in a timely manner.

7. Pre-training assessment: Schedule and administer cognitive, behavioral and electrophysiological assessments. These included: ATEC, Vineland, SRS, QEEG, MSI, emotion discrimination, and neuroimaging

- *Autism Treatment Evaluation Checklist (ATEC),* a parental checklist is used to evaluate ASD treatment paradigms based on four subscale scores (speech/language/communication, sociability, sensory/cognitive awareness, health/physical/behavior) and a total score, which are weighted according to the response and the corresponding subscale.
- The *Vineland Adaptive Behavior Scales* is a valid and reliable test to measure a person's adaptive level of functioning. The content and scales are organized within a three-domain structure: Communication, Daily Living, and Socialization.
- The *Social Responsiveness Scale* (SRS) provides a quantitative metric of the type and severity of impairments in social functioning that are characteristic of ASD children with five subscales (receptive, cognitive, expressive, and motivational aspects of social behavior plus autistic preoccupations).
- *Quantitative EEG*. High-density recordings of the brain's electrical activity are measured and quantified to guide neurofeedback treatment. Participant's EEG are recorded in two conditions of eyes open and eyes closed. Conditions are randomly presented for a total of 9-10 minutes per condition. QEEG analysis involves comparison with a normative database and helps to identify the sites of greatest atypical EEG.
- *Mu Suppression Index (MSI)***.** The MSI was developed by Oberman (Oberman et al., 2005), and is used to assess changes in mu power in response to the observation of movement. The standard protocol is followed (i.e., participants view silent action videos, 120 s, on a computer monitor while counting the number of pauses in the action). The test consists of a baseline condition and three experimental conditions.
- *Emotion Discrimination Task (EDT). A* modification of the Eyes task described by Baron-Cohen et al. (Baron-Cohen et al., 1997) has been designed to assess ASD responses to faces and the emotions expressed by such stimuli, as such processing is atypical in ASD (Chawarska & Shic, 2009; Scherf et al., 2008; Hadjikhani et al., 2007). The images are preceded by a fixation cross for 2 seconds. A cue photo of the eye region of a face appears at the top of the screen for several seconds. Words at each corner appear along with the image, and the participant's task is to pick which word describes the emotion being expressed.
- Neuroimaging. We made excellent progress in terms of gathering the neuroimaging data before and after training and beginning the analyses to determine whether NFT induced neural plastic changes and affected the brain's functional neuroanatomy. Some participants required training with a mock scanner to desensitize them to the scanning equipment and some participants exhibited significant movement artifact as to necessitate excluding them from the

analysis. The entire imaging session lasted approximately 1.5-hrs per participant and involved the following:

- 6-min echo planar imaging (EPI) resting state scan
- Three 5-min EPI task runs (the task is an imitation task)
- A spoiled gradient recalled (SPGR) high-resolution anatomical scan
- 8-min diffusion tensor imaging scan
- 3-slice magnetic resonance spectroscopy (MRS) scan

8. QEEG-guided neurofeedback training: Schedule and begin 30 hrs of training. This will require setting up parking areas for participants, arranging for a student to act as scheduler and point of contact with parents.

All participants received 90 minutes of neurofeedback training each week. They were trained for 20 weeks for a total of 30 hours of training. The recording sites used for initial training was always the C4 electrode site. Neurofeedback involved recording EEG activity and using the modulation of that activity to control aspects of a video game or to control playing of a DVD movie. The approach required keeping power in the EEG rhythm in the 8-13 Hz range above a pre-determined threshold while keeping EMG activity and other specified EEG frequencies (namely theta and gamma) below pre-defined thresholds. On the screen participants saw a display of at least three threshold bars alongside the video game window. One corresponded to the rewarded frequency and the other two correspond to inhibited frequencies. Rewards (i.e., if the video game was a racing car, the car would move or a DVD movie would play if the thresholds were met) were given based on satisfying two conditions: 1) power in appropriately identified band exceeded a specified threshold, and 2) power from the theta (related to blinks) and high frequency gamma (related to muscle movement) activity was inhibited if above a specified threshold. Theta and gamma inhibition feedback was included in the design for two reasons. First, it ensured that individuals in the experimental group could not advance in the game or play the DVD by producing movement-induced power increases in the entire EEG spectrum. Second, it allowed us to distinguish improvement effects as a function of EEG modulation, modulation of autonomic nervous system activity, or placebo effects.

If participants met the clinical criteria and completed the EEG and neuroimaging components then they were ready to begin the neurofeedback training. This required a great deal of flexibility on our part to be able to accommodate children who were very busy during the school year and summer. We found that an important component of this was to have one person entirely responsible for scheduling, as there was a significant amount of changes during the first few weeks. Once a schedule was found that worked for parents and children it was usually kept throughout the rest of the training.

9. Post-training assessment: Following completion of 30 hrs of training, begin scheduling and administration of cognitive, behavioral, and electrophysiological assessments. These are the same tools used in the pre-training assessment (ATEC, Vineland, SRS, QEEG, MSI, emotion discrimination, and neuroimaging).

An early problem was paying a higher rate for scanner time. However, undoing a re-budgeting effort and returning to the original budget proposed for the project corrected this. The entire imaging session lasted approximately 1-hr per participant and involved the following:

- 6-min echo planar imaging (EPI) resting state scan
- Three 5-min EPI task runs (the task is an imitation task)
- A spoiled gradient recalled (SPGR) high-resolution anatomical scan
- 8-min diffusion tensor imaging scan
- 3-slice magnetic resonance spectroscopy (MRS) scan

Post-training assessments also required a great deal of scheduling and flexibility to make sure they were completed within a reasonable amount of time. We worked out a system for scheduling all the assessments such that it only required two additional visits once training was completed.

10. Data processing and analysis

A significant amount of progress was made in processing and analyzing data collected, both in terms of behavioral, electrophysiological, and functional neuroanatomical results (see Appendix).

11. Preparation and submission of journal article(s)

We published one full-length journal article, submitted one, and are completing a third manuscript. We have also submitted a book chapter, presented at three professional symposia, and submitted 3 abstracts (see Reportable Outcomes).

12. Integration of project findings & preparation of R01 application

We are in the process of preparing an NIH R01 grant proposal to be submitted October 2013.

KEY RESEARCH ACCOMPLISHMENTS:

- An additional research center, the Speech and Language Development Center (SLDC) in Buena Park, CA, was established to allow for increased accrual of participants.
- Personnel at SLDC who administered clinical assessments were identified, trained and certified. Paper and pencil assessments, software tools, and protocol design were set up at SLDC.
- SLDC Research assistants who administered and scored outcome measures, electrophysiological tests and neurofeedback training were trained successfully in a reasonable amount of time.
- HRPO approval was obtained
- Intense recruitment efforts were slightly behind schedule given the long delay to get the project started
- Mechanisms for recruiting, selecting, and assessing participants were put in place and working relatively quickly
- Hardware and software for the neurofeedback training at UCSD and SLDC were acquired, piloted, and were tested in a short period of time
- Process of training Research Assistants was successful and efficient

- Protocols and procedures for the neuroimaging part of the project were in place and tested successfully in a short period of time
- Participant recruitment was on target
- Participants who completed training was only slightly below expectations
- Some participants were initially recruited but chose to drop out or were excluded due to problems (12 ASD and 5 TD)
- Neuroimaging scans were completed for all pre-training participants
- Neuroimaging scans were completed for all post-training participants
- Preliminary analysis of functional neuroanatomy was consistent with predictions
- Neurofeedback hardware and software implementation, protocols, testing of procedures, and training of personnel are completed and we have begun testing participants.

REPORTABLE OUTCOMES:

1. Pineda, J.A., Juavinett, A., and Datko, M. Self-regulation of brain oscillations as a treatment for aberrant brain connections in children with autism. Medical Hypothesis, 79: 790-798 (2012). [Full length manuscript]
2. Pineda, J.A., Carrasco, K., Datko, M., Pillen, S. and Schalles, M. Neurofeedback training produces normalization in behavioral and electrophysiological measures of high functioning autism. To be published in Mirror Neurons: Fundamental Discoveries, Theoretical Perspectives and Clinical Implications, Phil. Trans. B. Editors: P.F. Ferrari and G. Rizzolatti. [Full length book chapter]
3. Datko, M., Müller, R-A., Pineda, J.A. Functional neuroanatomical changes produced by mu-based neurofeedback training in children on the autism spectrum, in preparation. [Full length manuscript]
4. Fishman, I., Keown, C.L., Lincoln, A., Pineda, J.A., Mueller, R-A. Functional connectivity of two key social brain networks in autism spectrum disorder. Submitted [Full length manuscript]
5. Datko, M. C., Pineda, J. A., Müller, R. A. Functional Neuroanatomical Changes Induced by Mu-based Neurofeedback Training in Children on the Autism Spectrum, IMFAR, Toronto, Canada, 2011. [Abstract]
6. Pineda, J.A., Datko, M. & Axel-Muller, R. Functional Neuroanatomical Changes Induced by Mu-based Neurofeedback Training in Children on the Autism Spectrum. CNS Conference, Chicago, 2012. [Abstract]
7. Datko, M., Pineda, J. and Mueller, RA. Neurofunctional and behavioral changes following mu neurofeedback training in children on the autism spectrum. SFN Abstract, 2013 [Abstract]
8. Datko, M., Carrasco, K., Müller, R-A. , Pineda, J.A. Changing the Dynamics of the Mirror Neuron System through Neurofeedback: Effects on ASD Behavior, Electrophysiology, and Functional Neuroanatomy, SABA presentation, 2012. [Symposium presentation]
9. Pineda, J.A. Mirror Neurons, Mu Rhythms and Autism Spectrum Disorders. Developmental Psychobiology Conference, Hawaii, 2012. [Symposium presentation]

10. Pineda, J.A. Functional Neuroanatomical Changes in the Mirror Neuron System Produced by Neurofeedback Training of Children on the Autism Spectrum. Mirror Neuron Conference, Erice, Italy, 2012. [Symposium presentation]

CONCLUSION:

It is widely agreed that the autistic brain is characterized by widespread aberrant connectivity that could underlie abnormal social behaviors. Nonetheless, the nature of the brain's experience-dependent plasticity suggests that these abnormal connection patterns may be reversed with the proper type of treatment. Indeed, neurofeedback training (NFT), an intervention based on operant conditioning resulting in self-regulation of brain electrical oscillations, has shown promise in addressing marked abnormalities in functional and structural connectivity. The effects of 30 hours of mu-rhythm based NFT on children on the autism spectrum (ASD), as well as typically developing (TD) children, were assessed behaviorally, electrophysiologically, and with fMRI. Prior to training, ASD participants showed significantly less activation compared to TD controls in an object-directed imitation task in areas associated with the human mirror neuron system (hMNS). Following training, ASD participants showed significantly greater activation in this task while TD participants did not. Prior to training, ASD participants also showed both over- and underconnectivity in resting state functional connectivity between areas of the hMNS compared to TD participants. These differences were significantly reduced following NFT. The fMRI changes accompanied improvements in behavioral and electrophysiological assessments in the ASD but not TD participants, indicating that the positive benefits shown to result from NFT are accompanied by modifications in functional neuroanatomy.

An obvious limitation to these data is the small sample size. However, the results are surprisingly strong and clear in light of this caveat. Furthermore, it provides a very good preliminary set of results to leverage for an R01 grant. We anticipate a number of future analyses to be performed on the data obtained from this study. One important step will be to assess the degree of correlation between symptom severity and the magnitude of behavioral/neural response to NFT. This type of analysis will address the issue of whether mu neurofeedback is more beneficial for individuals with more severe symptoms of ASD. Another important question is whether those individuals with the most dramatic behavioral and parental assessment improvements also show the greatest changes in functional neurophysiological activation during the imitation task.

Our findings have implications for operationalizing the benefits of NFT towards practical solutions to the early diagnosis and possible repair of MNS deficits in autism. It is generally accepted that autism research should be translational. The increasing number of families affected by it represent a growing concern for society, with therapeutic approaches limited in efficacy but costly in time and money. Despite solid evidence for the importance of genetic factors in autism, numerous large-scale studies have failed to translate this into effective therapies. The reasons are many and imply that a comprehensive model of neurodevelopmental disturbances in autism may not become available for decades. However, children with ASD and their parents cannot wait, but rely on the immediate pursuit

of treatment options that are promising. Techniques such as EEG have been used extensively to characterize functional brain abnormalities associated with ASD. While the current project will significantly contribute to this endeavor, its primary impact and importance derives from the application of these techniques to prediction and intervention. Cognitive, behavioral, and electrophysiological characterization before and after neurofeedback training allowed us to develop phenotypic responses and therefore predictive measures to assess the success or failure of such interventions. NFT approaches appear to hold great promise for a large portion of children with ASD and this approach is particularly promising because it could combine QEEG analyses with an easy to use, ASD targeted in-home neurofeedback system. We believe that with such an approach many of the barriers to successful therapeutic intervention can be overcome. QEEG can be used to both identify ASD children likely to benefit from training, and profile the training protocol most likely to benefit an individual child. The in-home system provides an autism-specific, easy to use, comfortable therapeutic option that has the potential to improve compliance and the quality of data used for the feedback, increasing overall efficacy. Providing a personalized approach to this type of intervention, which can be conducted primarily in the home, will substantially reduce costs in time and money for ASD families, while improving the potential for positive behavioral outcomes seen previously in laboratory studies.

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APPENDICES:

Personnel involved in project

Jaime A. Pineda, Ph.D., Principal Investigator
Ralph-Axel Mueller, Ph.D., Co-Investigator (SDSU subcontract)
Dinesh Shukla, Ph.D., Postdoctoral Fellow (SDSU subcontract)
Michael Datko, Graduate Student Researcher

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1. IMFAR, Toronto, Canada, 2012

Functional Neuroanatomical Changes Induced by Mu-based Neurofeedback Training in Children on the Autism Spectrum. Datko, M. C.[1,2], Pineda, J. A.[1], Müller, R. A.[2]

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Background: Autism Spectrum Disorders (ASD) may arise from atypical anatomical and functional connections and therefore have been characterized as a 'disconnection syndrome'. Impaired connectivity may lead to desynchronization and ineffective intra- and interhemispheric communication in neural circuits affecting higher order cognitive processes. While no single explanation can account for the ASD profile, converging evidence implicates the human mirror neuron system (MNS). Studies from our laboratory have shown that ASD individuals exhibit normal EEG mu rhythm suppression for self-generated movement but fail to suppress during observation of movement compared to typically developing (TD) controls. On the other hand, suppression is normal if the actors being observed are familiar, suggesting that the MNS is not entirely broken. We have shown that significant improvement occurs in social engagement and related behaviors, as well as in the electrophysiology of ASD children following neurofeedback training focused on the mu-rhythm.

Objective: The present study tested whether functional and structural neuroanatomical changes occur after 20 weeks of mu-based neurofeedback training.

Methods: Neurofeedback training is an operant conditioning task in which trainees learn to control mu rhythm (8-13 Hz) power at electrode site C4, over the sensorimotor cortex in the right hemisphere. Games and movies on a computer reward increased mu-power and decreased muscle activity. All participants complete 30 hours of this training (45 min/session x 2 sessions/week x 20 weeks). Prior to and again immediately following training, participants underwent fMRI scans that included the following protocols: resting state fMRI (6 min), 3 fMRI runs of a task that involved imitation and observation of object-oriented finger movements (total of 15 min), anatomical (5 min), and diffusion tensor imaging (10 min). Contrary to imitation tasks previously used by Iacoboni (1999) and Williams (2006), the imitation task in our study was object-oriented (pressing buttons on a button-box). Diffusion tensor imaging data were collected to assess white matter changes associated with neurofeedback in pathways connecting areas of the MNS.

Results: Before training, greater activation occurred in regions of interest related to MNS in TD compared to ASD during object-oriented imitation and observation. These areas of differential activation included left inferior frontal gyrus (IFG) and bilateral inferior parietal lobules. Abnormal

resting state functional connectivity (both under- and over-connectivity) between MNS regions of interest was also seen in ASD compared to TD groups. Altering mu rhythm dynamics with training was found to result in increased activity in the IFG and other relevant MNS areas, as well as normalization of functional connectivity in the MNS circuits in ASD children.

Conclusions: These preliminary data indicate plasticity within the mirror neuron system occurs in response to mu-based neurofeedback training in ASD. Both activation and connectivity measures were found to normalize with training.

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2. CNS Conference, Chicago, 2012.

Functional Neuroanatomical Changes Induced by Mu-based Neurofeedback Training in Children on the Autism Spectrum. Pineda, J.A.[1,2], Datko, M.[1] & Axel-Mueller, R.[3] Cognitive Science Department[1] and Group in Neurosciences[2], UCSD, Psychology Department[3], San Diego State University, San Diego, CA.

Autism Spectrum Disorders (ASD) may arise from atypical anatomical and functional connections and therefore produce abnormal activity among different regions of the brain. This type of 'disconnection syndrome' could lead to desynchronization and ineffective intra- and interhemispheric communication in neural circuits affecting higher order cognitive processes. While no single explanation can account for the ASD profile, converging evidence implicates the human mirror neuron system (MNS). Studies from our laboratory have shown that ASD individuals exhibit normal EEG mu rhythm suppression for self-generated movement but fail to suppress during observation of movement compared to typically developing (TD) controls. On the other hand, suppression is normal if the actors being observed are familiar, suggesting that the MNS is not entirely broken. We have shown that significant improvement occurs in social engagement and related behaviors, as well as in the electrophysiology of ASD children following neurofeedback training focused on the mu-rhythm. The present study tested whether functional neuroanatomical changes occur after mu-based neurofeedback training (45 min x 2 week x 20 weeks). Before training, greater activation occurred in regions of interest related to MNS in TD compared to ASD during object-oriented imitation and observation. Abnormal functional connectivity, under- and over-connectivity, were also present in ASD compared to TD groups among MNS areas. Altering mu rhythm dynamics with training was found to result in increased activity in the inferior frontal gyrus (IFG) and other relevant MNS areas, as well as normalization of functional connectivity in the MNS circuits in ASD children.

3. Society for Neuroscience (SFN), San Diego, 2013

Neurofunctional and Behavioral Changes following mu neurofeedback training in children on the autism spectrum. Datko, M.; Pineda, J.; Mueller, RA.

Objectives: Autism spectrum disorders are characterized in part by socio-communicative impairments that are resistant to many forms of treatment. These impairments may be due in part to aberrant connections within the human action-observation or mirror neuron system (MNS). A putative marker of MNS activation is the 8-13 Hz sensorimotor EEG signal known as the mu rhythm, which does not

modulate during observation of biological actions in individuals with ASD. One proposed intervention to normalize MNS activity is neurofeedback training (NFT), an operant conditioning technique in which trainees learn to volitionally control power levels in EEG frequency bands based on real-time feedback from a computer. We used functional MRI to compare the effects of mu-rhythm based NFT in a group of children and adolescents with high-functioning ASD and a matched group of typically developing (TD) children.

Methods: All participants completed between 20-30 hours of NFT, during which participants attempted to modulate mu EEG power (8-13 Hz) at electrode site C4 to control aspects of a video game or movie. The goal was to maintain mu power above a pre-determined threshold while keeping theta and beta power below pre-determined thresholds. All participants received fMRI scans before and after training in which they performed an action imitation task, a resting state scan, and anatomical and diffusion tensor imaging scans. Along with parent questionnaires and assessments related to ASD symptom severity and socio-communicative abilities, data from these pre- and post-NFT scans were used to measure effects of the mu NFT.

Results: Brain activation data, as measured by the percent BOLD signal change, was measured during an action observation and imitation task before and after NFT. These data was used in a whole brain ANOVA including group (ASD or TD) as a between-subject factor and training (pre-NFT or post-NFT) as a within-subject factor. Statistically significant group x training effects were found in brain areas comprising the MNS. In the ASD group, these changes in BOLD activation following the training were accompanied by positive changes in pen-and-paper assessments of socio-communicative abilities.

Conclusions: Mu NFT has positive benefits for socio-communicative impairments in ASD, and these behavioral benefits are accompanied by increased blood flow activations in brain areas involved in action observation and imitation. Mu NFT appears to have different effects on a matched group of typically developing individuals, who showed minimal changes in behavioral measurements and a decrease in imitation-related brain activations following NFT. Our findings suggest that mu NFT could be a useful adjunct to ASD treatment.

4. Full-length manuscript, in preparation (an early draft).

Functional Neuroanatomical Changes Produced by Mu-based Neurofeedback Training in Children on the Autism Spectrum

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Abstract

It is widely agreed that the autistic brain is characterized by widespread aberrant connectivity that could underlie abnormal social behaviors. Nonetheless, the nature of the brain's experience-dependent plasticity suggests that these abnormal connection patterns may be reversed with treatment. Indeed, neurofeedback training (NFT), an intervention based on operant conditioning resulting in self-regulation of brain electrical oscillations, has shown promise in addressing marked abnormalities in functional and structural connectivity. In the present study, the effects of 30 hours of mu-rhythm based NFT on children on the autism spectrum (ASD), as well as typically developing (TD) children, were assessed behaviorally and with functional MRI. Significant interactions between group status (ASD vs. TD) and training status (pre vs. post) were found for fMRI BOLD signal activations during an object-directed imitation task. These effects were driven in part by improved activations in the ASD group following NFT. Prior to training, ASD participants also showed both over- and underconnectivity in resting state and task-regressed functional connectivity between areas of the human mirror neuron system (hMNS) compared to TD participants. These differences were significantly reduced following NFT. The fMRI changes accompanied improvements in behavioral assessments in the ASD but not TD participants, indicating that the positive benefits shown to result from NFT are accompanied by modifications in functional neuroanatomy.

Introduction

Numerous findings support the hypothesis that social deficits in autism are the result of abnormal connectivity between brain regions associated with social cognition and action perception {mueller2007}. From these findings an underconnectivity hypothesis of ASD has emerged positing that "autism is a cognitive and neurobiological disorder marked and caused by under functioning integrative circuitry that results in a deficit of integration of information at the neural and cognitive levels" {just2004}. A more general hypothesis has also been proposed suggesting that ASD may involve "local overconnectivity AND long-range underconnectivity" {courchesne2005}. Additionally, previous studies indicate that individuals with ASD show decreased resting state connectivity in the default mode network (DMN) compared to typically developing (TD) controls {cherkassky2006}, as well as a reduced "switching" from this network to task-related networks during task performance {kennedy2008}. ASD individuals do not show as much deactivation, relative to controls, of the DMN when engaged in a task, a finding that indicates problems with regulation of functionally connected networks {kennedy2006}.

In particular, connectivity and activation abnormalities have been observed in brain regions constituting the human mirror neuron system (hMNS). The hMNS has provided a potential neurobiological substrate for understanding many key concepts in human social cognition directly relevant to the behavioral and cognitive deficits observed in ASD {williams2001}, including the ability to comprehend actions, understand intentions, and learn through imitation. First described in single-unit recordings by Rizzolatti and colleagues in the macaque monkey {dipellegrino1992}, mirror neurons are involved in both self-initiated action and the representation of action performed by others. Neurons in the pars opercularis of the inferior frontal gyrus (IFG) and in the inferior parietal lobule (IPL) show increased firing while executing and observing the same action, representing a potential mechanism for mapping seeing into doing {rizzolatti2004, pineda2005}. Cells that fire preferentially in response to actions and to the observation of actions have also been observed in the human brain using single unit recordings {mukamel2010}. Indeed, a homologous network also including the posterior superior temporal sulcus (pSTS) has been described in humans using fMRI {iacoboni1999}.

Although some studies have raised questions about the role of mirror neurons in human social behavior {hickock2009, turella2009}, an increasing amount of work suggests that a dysfunction in the hMNS does contribute to social deficits

{nishitani2004,hadjikhani2006,bernier2007,dapretto2006,theoret2005,oberman2005}. Individuals with ASD have marked impairment in social skills, from joint attention to understanding the intentions of others or "mind-blindness" {carpenter1998,baroncohen2009}, and as has been noted in a number of recent reviews, deficits in hMNS activity may explain the poor socialization skills prevalent in the disorder {mnsasdreview}. A particularly relevant fMRI study {dapretto2006} demonstrated decreased activation in the pars opercularis of the IFG in autistic individuals during imitation of facial expressions, and found that activity in this region was inversely related to symptom severity in the social domain. EEG studies have shown that putative electrophysiological biomarkers of hMNS activity also show abnormalities in ASD {bernier2007,oberman2005,oberman2008,raymaekers2009}. Indeed, a body of evidence links the spectral dynamics of an EEG signal known as the mu rhythm to the functioning of the hMNS {pineda2005}. Particularly relevant are scalp-recorded EEG patterns of activity in the alpha mu (8-13 Hz) and beta mu (15-25 Hz) range that are most evident over the central region of the scalp overlying the sensorimotor cortices and that are modulated by motor activity {altschuler1998}. Relative to baseline, mu power is suppressed, not only during actual execution, but also during the observation and imagination of body movements {pfurtscheller2006,muthukumaraswamy2004,mcfarland2000}. In a recent study, Keuken et al {keuken2011} showed that repetitive transcranial magnetic stimulation (rTMS) to disrupt the function of left IFG in normal human adults increases reaction times during an emotion recognition task and eliminates the suppression of the mu rhythm. In another recent study, Arnstein used fMRI and EEG to show that suppression of mu power is correlated with BOLD signal activations in areas associated with the hMNS {arnstein2011}. In ASD, this mu rhythm suppression is absent during observation of body movements, supporting the role of an altered MNS in the disorder {oberman2005,oberman2007}.

There is evidence from earlier studies that neurofeedback training (NFT) produces positive behavioral changes in children with ASD by normalizing the aberrant connections within and between neural circuits. Neurofeedback is a form of operant learning in which participants develop implicit control over the frequency-based spectral dynamics of scalp-recorded electrical cortical oscillations. Over time, participants develop strategies, implicitly or explicitly, to control a visual representation of frequency power levels. The best-established clinical application of the use of NFT and operant conditioning is arguably the treatment of epilepsy {sterman1996} via conditioning of the "sensorimotor rhythm" or SMR, described initially by Sterman and colleagues {roth1967}. The SMR is an EEG oscillation with a frequency of 12–20 Hz, similar to EEG sleep spindles. During the testing of a highly epileptogenic compound, Sterman and colleagues found elevated seizure thresholds in cats that had previously taken part in SMR conditioning, suggesting that the SMR training itself had predisposed the cats against experiencing seizures. These findings have been successfully extrapolated to humans where it has been documented that seizure incidence is significantly lowered through SMR training {walker2008}. In line with the work by Sterman and colleagues, Ros et al. {ros2010} have shown that self-regulation of EEG rhythms in quietly sitting, naive humans significantly affects the subsequent corticomotor response to transcranial magnetic stimulation (TMS), producing durable and correlated changes in neurotransmission. Finally, Beauregard and Lévesque J. et al. {beauregard2006} scanned 15 unmedicated children with ADHD randomly assigned to an experimental group that received NFT, and five other ADHD children assigned to a control group that did not receive NFT while they performed a Counting Stroop task. Prior to training, a significant focus of activation occurred in the left superior parietal lobule for both groups but no activation in the anterior cingulate cortex (ACC). Following training, there was still increased activation of the left superior parietal lobule for both groups, but for the experimental group only there was a significant activation of the right ACC.

NFT requires less time to be efficacious than other behavioral interventions and produces fewer side effects than pharmacotherapies {fuchs2003,fox2005}. In a recent review of the literature, Coben et al. {coben2009} argued that while further research is necessary, a variety of studies support a Level 2 determination ("possibly efficacious") for the application of neurofeedback for autistic disorders. A

previous mu-NFT study in children with ASD showed improvements in sociability and attention, and a normalization of action-observation-related mu-rhythm suppression that is normally absent {pineda2008}. Therefore, it is hypothesized that lasting neuroplastic changes can result from repeatedly engaging circuits involved in developing and sustaining volitional control of cortical oscillations. Furthermore, it was hypothesized that changes in mu rhythm and associated brain circuits would be more pronounced in the ASD group, where these dynamics are compromised, compared to the TD group.

To test this hypothesis, we examined the effects of 20-30 hours of mu-NFT on a group of high functioning ASD children and a comparison group of typically developing (TD) children between the ages of 8-17 years. Training was administered in 45- or 60-minute sessions 1-2 times per week over the course of 20-30 weeks. Groups were matched for age, handedness and intelligence.

To investigate the effects of training on functional activations of the hMNS, subjects had functional MRI scans while performing an imitation task that was designed to elicit activity specifically in the hMNS. We adapted an imitation task first used to study hMNS activation in healthy adults by Iacoboni et al {iacoboni1999}. Previous versions of this task have elicited activation in two areas associated with the hMNS: the inferior parietal lobule and superior temporal sulcus. The task has also been used to show activation differences between TD and ASD groups in these two areas.

We predicted that, prior to NFT, brain activation during the imitation task would be significantly lower in the ASD group compared to the TD group, and that this between-group difference would be localized primarily in areas associated with the hMNS. We also predicted that following NFT, hMNS activation differences between these groups would be reduced. Additionally, we predicted that autistic symptom severity would be reduced following training, and that this reduction would correlate with changes in BOLD fMRI measurements. Finally, we predicted that any functional neuroanatomical effects resulting from the training would be more significant in participants whose assessment profile reflected greater symptoms of autism.

Methods

Participants.

7 high-functioning (age 7 - 17; M = 13.51 + 1.33 years; 5 male) and 7 typically developing (age 7 - 17; M = 11.18 + 1.12 years; 5 male) subjects were included in the study. Participants were recruited from San Diego and Los Angeles Counties via local support groups for children with ASD and other disabilities, from local schools and recruitment posters, and via Valerie's List, a San Diego Internet autism support group. All but one participant in the ASD group had their diagnosis verified by a clinician using the ADOS, ADI, and WASI and met the criteria of high functioning autism with an IQ \textgreater 80. Participants and parents gave informed assent and consent, respectively. The University of California, San Diego's Institutional Review Board approved the study. Subjects' demographic information is summarized in Figure \ref{fig:demopre} (located at bottom of paper).

Neurofeedback Training

All participants received 45 minutes of training twice per week for 20 weeks for a total of approximately 30 hours. Training involved recording EEG activity from the C4 electrode site and using the modulation of that activity to control aspects of a video game or control playing of a DVD movie. Both approaches required keeping the EEG rhythms in a specific frequency range above a pre-determined threshold while inhibiting other specified EEG frequencies (theta and gamma) and keeping them below a pre-defined threshold. On the screen participants saw a display of at least three threshold bars alongside the video game window. One corresponded to the rewarded mu frequency and the other two corresponded to the inhibited frequencies. Rewards (e.g., if the video game is a racing car, the car will move or a DVD movie play if the thresholds are met) are given based on satisfying two conditions: 1) power in the specified frequency (8-13 Hz mu band) exceeds a specified threshold, and 2) power from the theta

(related to blinks) and high frequency gamma (related to muscle movement) activity is inhibited and falls below a specified threshold. Theta and gamma inhibition feedback was included in the design for two reasons. First, they ensured that individuals in the experimental group could not advance in the game or play the DVD by producing movement-induced power increases in the entire EEG spectrum. Second, they allowed improvement effects as a function of EEG modulation, modulation of autonomic nervous system activity, or placebo effects.

Behavioral Assessments

In addition to diagnostic interviews for ASD participants, two pen-and-paper questionnaires were given to parents of each participant before and after training. Specifically, the Autism Treatment Evaluation Checklist (ATEC) and the Social Responsiveness Scale (SRS) were used. These assessments include multiple dimensions, or categories, of symptoms. Each subject's score on each dimension was calculated as a percentage of the highest possible score for that dimension. The dimensions were analyzed using a repeated measures ANOVA with training (pre-, post-) and dimensions as a within subjects factor and group (control, experimental) as a between subjects factor. The Autism Treatment Evaluation Checklist (ATEC) is a parental checklist to evaluate ASD treatment paradigms based on four subscale scores (speech/language/communication, sociability, sensory/cognitive awareness, health/physical/behavior) and a total score, which are weighted according to the response and the corresponding subscale. Each category contains multiple symptoms that are each rated on a scale of 1-5. Each subject's score on each dimension of the ATEC will be calculated as a percentage of the highest possible score for that dimension. The Social Responsiveness Scale (SRS) provides a quantitative metric of the type and severity of impairments in social functioning that are characteristic of ASD children with five subscales (receptive, cognitive, expressive, and motivational aspects of social behavior, plus autistic preoccupations), administered by research staff with the child.

fMRI Data Collection

All MRI data were acquired on a GE 3T MR750 scanner with an 8-channel head coil. High-resolution structural images were acquired with a standard FSPGR T1-weighted sequence (TR: 11.08ms; TE: 4.3ms; flip angle: 45°; FOV: 256mm; 256 x 256 matrix; 180 slices; 1mm^{3} resolution).

Data for the imitation task were acquired in three functional runs, each consisting of 150 whole-brain volumes acquired in 42 interleaved slices using a single-shot, gradient-recalled, echo-planar pulse sequence (TR: 2000ms; TE: 30ms; flip angle: 90°; 64 x 64 matrix; 3.2mm slice thickness; in-plane resolution 3.4mm^{2}).

Cardiac and respiratory data were collected for each participant during each functional run. These data were used to create regressors for removing physiological noise from the functional data.

Preprocessing and analysis of fMRI data

All data were preprocessed and analyzed using the Analysis of Functional Neuroimages suite {cox1996}. The first four time points for each run were discarded to remove effects of signal instability, and slice-time correction was performed. Functional and resting state data were co-registered to Talairach space (and specifically to the TT_N27 "Colin Brain" packaged with the AFNI software), resampled to isotropic 3 mm^{3} voxels, and spatially smoothed to a global full-width at half-maximum (FWHM) of 6mm.

Conventional motion correction was performed for all functional scans. Additionally, considering the known impact of head motion on BOLD signal correlations in functional connectivity analyses {power2012,vandijk2012}, further steps beyond conventional motion correction were taken. Six rigid-body motion parameters, estimated based on realignment of functional volumes, were modeled as nuisance variables and their contribution to the overall signal was removed via linear regression. Time points with excessive motion (defined as motion exceeding 1.5mm displacement from one time point to the next), along with the following 10 time points, were censored so as to exclude

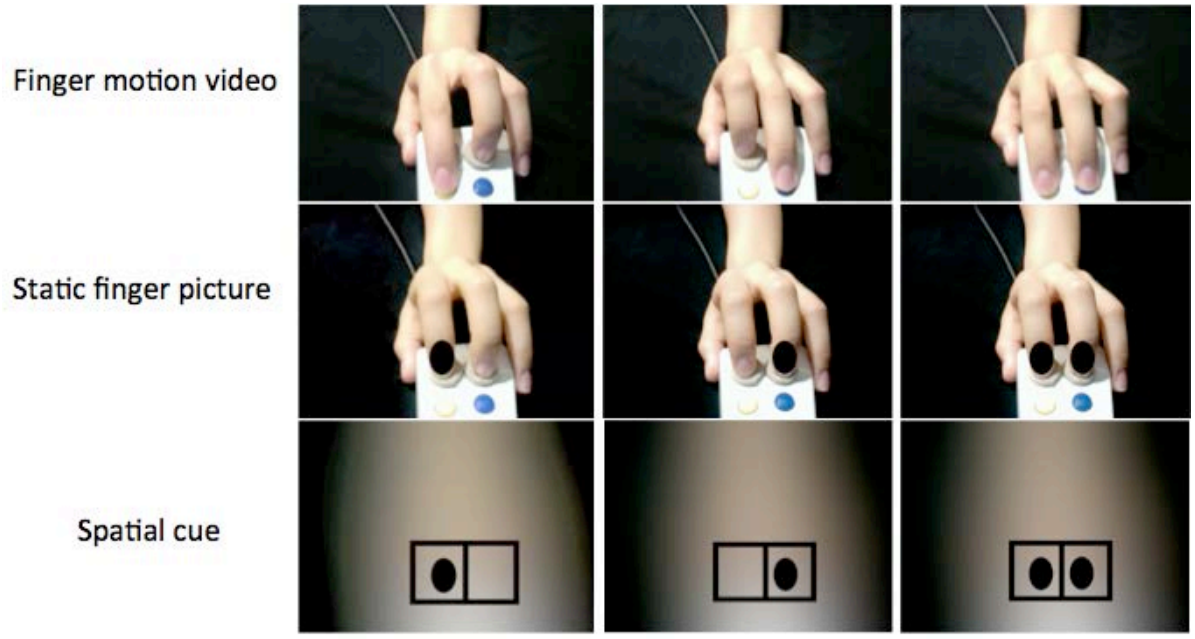
them from the final statistical analysis. An average of approximately 13 percent of the total time points were censored, and this number did not differ significantly between groups or before/after the NFT.

Functional MRI data from each of the three task runs for each participant were then scaled to a mean of 100, and concatenated to create a single time-series. The hemodynamic impulse response function (IRF) for each stimulus type was estimated using a general linear model. The hemodynamic response function for each condition block was estimated using a simple 27-second block function.

Imitation Task

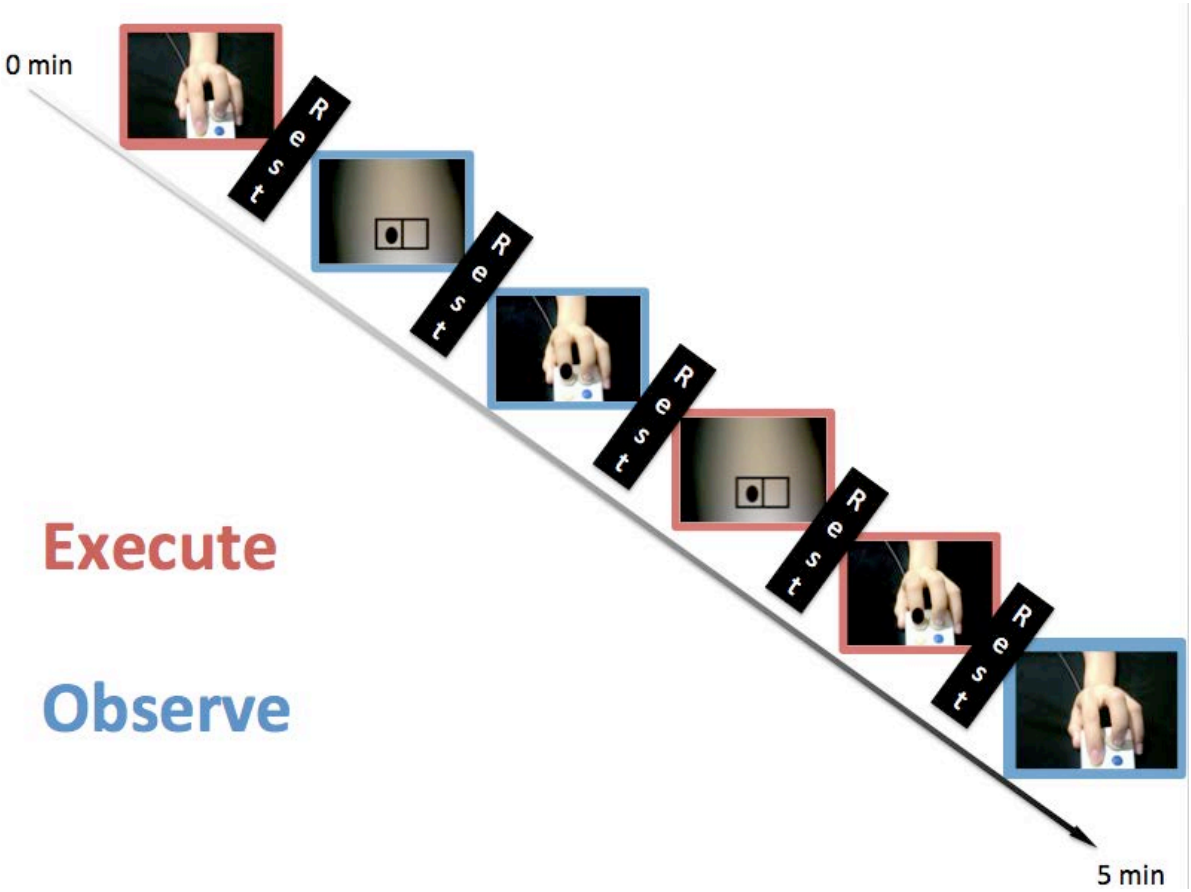
We tested the prediction that NFT would produce changes in functional brain activation by adapting an imitation task first used to study hMNS activation in healthy adults by Iacoboni et al (15). A similar task was also used by Williams et al (48) to reveal hMNS activation differences between participants with ASD and healthy controls. The task in the present study required each participant to watch movies and pictures of a hand and to make responses based on observed finger movement or other visual cues.

The task included six distinct conditions plus a rest baseline. In one condition, participants were instructed to imitate movements of fingers shown in a short clip of a hand pressing one or both buttons on a button box. The box shown in the videos was the same one on which subjects made their responses in the scanner. In another condition, participants were also instructed to press either or both of the buttons on their button box; however, the stimuli was not a motion video but rather a still image of the hand from the videos, with a black dot in front of either or both of the fingers as an indicator of which button to press. In a third condition, a blurred background with luminosity resembling that of the hand picture was shown, with black dots within a rectangle as the indicators of which button the subject should press. In the other three conditions, the same three stimuli types were shown (motion videos of button pressing, static image of hand with dot in front of finger, and blurry background showing dots within a rectangle) but the subject was instructed to simply observe and pay attention to these rather than making any button-pressing responses. Examples of the stimuli are shown in Figure



The conditions were presented in a blocked design, alternating between 27-second blocks of one condition and 20-second blocks of rest. During each of the 27s task blocks, a total of 9 2.5-second stimuli were shown, with 0.5s pauses between these stimuli. Regardless of block type (video hand,

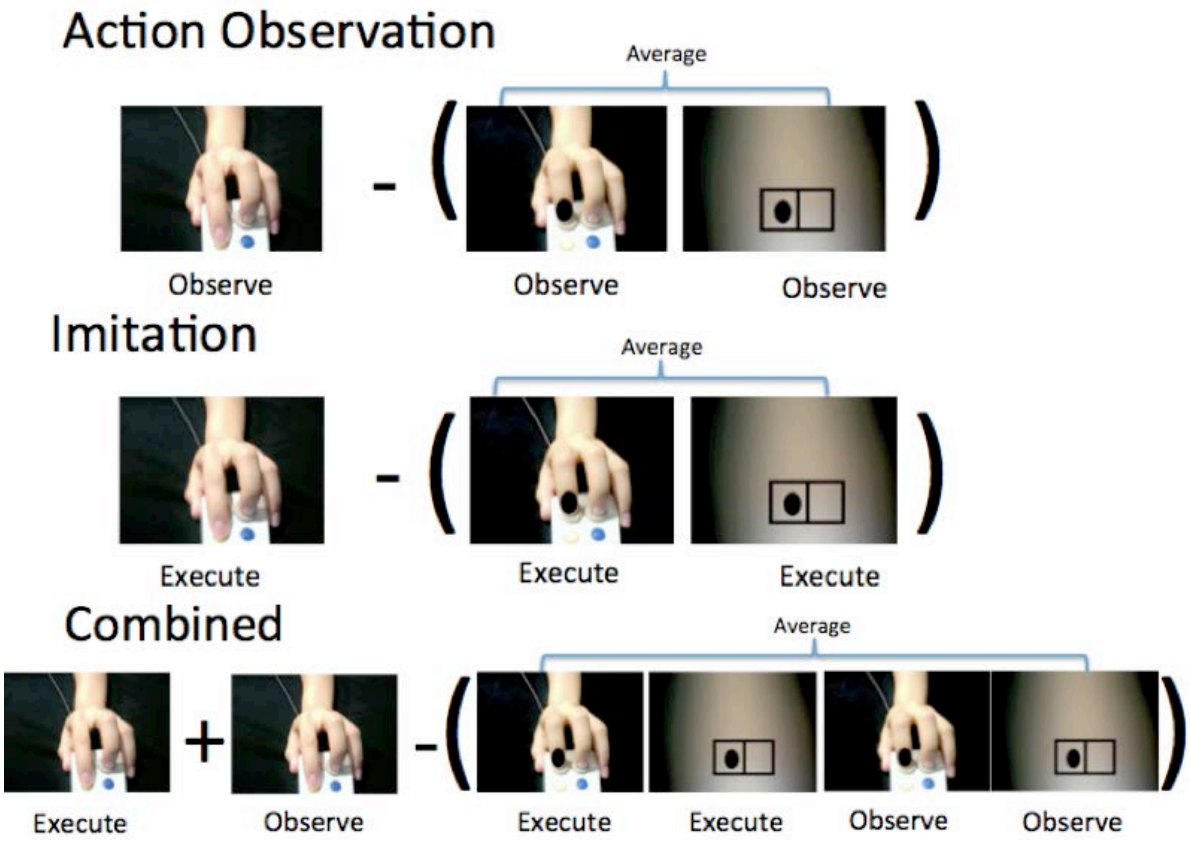
static hand, or spatial cue), each block showed 3 stimuli for a button press with the middle finger, 3 stimuli for the index finger, and 3 stimuli for both fingers simultaneously. Each of these stimuli was displayed 3 times per block, in semi-random order, for a total of 9 stimuli clips/pictures per block. During the pauses, a white fixation cross was displayed in the middle of a black background. The 20-second rest condition between blocks was simply a white fixation cross in the middle of a black background, identical to that displayed during the resting state scans. The task was divided into three 5-minute runs, with each run containing one block of each of the six condition types and six rest periods. The conditions were presented in a different, semi-random order for each run. A visual representation of the task design used in the present study is shown in Figure



BOLD signal activation analysis

By subtracting the activation that occurs while participants make a button-press response to the static hand pictures from that which occurs when they imitate the finger movement videos, the activation associated with the salient visual features of the hand is eliminated and what remains is activation resulting from observing, mirroring, and imitating the finger motion itself. Likewise, by subtracting the activation during the presentation of the spatial cue stimuli from the activation during the finger movement videos, any activity increase that results from simple visuospatial reasoning and the overall luminosity of the image is controlled for. Therefore, our analysis contrasts ensure that activity related to visual salience as well as simple spatial reasoning is controlled for, leaving only activity related to mapping observed movements onto one's own motor system, also known as mirroring. Thus, the task activation analysis utilizes weighted contrasts where the average of the activation during the static hand

and spatial cue stimuli is subtracted from activation during the finger motion video stimuli. These contrasts are presented visually in Figure



The analysis of functional activation of the BOLD signal during the imitation task began by removing the mean from the voxelwise timeseries, thus converting the resulting beta values of the general linear model into a measure of percent signal change. After scaling the BOLD signal in this way, the 3 task runs for each participant were concatenated into a single run.

For each participant's concatenated, scaled task run, the task was modeled and estimates of BOLD signal percent change for each condition and contrast were obtained using the AFNI command 3dDeconvolve. The same command was also used to simultaneously remove physiological nuisance variables (cardiac, respiratory, and 6 head motion parameter estimates), and was used to regress out signal from white matter and ventricular regions.

A series of 2-factor whole-brain ANOVAs were performed on the beta weights (representing percent signal change) from the linear model of the previous step. Group status (ASD or TD) and training status (pre-NFT or post-NFT) were the two main factors, with 2 levels each. Brain activation data from 7 subjects in each group, before and after the training, were included in the ANOVA. One of these ANOVAs was performed for activation data from just the imitation contrast, one was performed for just the action observation contrast, and one was performed for the combined imitation plus action observation contrast. The results of each of these whole-brain ANOVAs were then corrected for multiple comparisons, using false discovery rate (FDR) simulation, to a corrected p-value of 0.05.

The voxel clusters showing significant interactions in the ANOVA were then used as masks to extract beta values (representing percent change in the BOLD signal) for each individual for each

contrast of interest. These extracted beta values were used as factors in the covariate analysis described below.

In addition to these ANOVAs, a series of whole-brain t-tests were performed on the three task activation contrasts. For each of these three contrasts, a t-test was performed between the TD group versus the ASD group prior to the training. A test between these same groups was then performed for the post-training data. Another t-test compared the pre-training ASD group to the post-training ASD group, while another compared the pre-training TD group to the post-training TD group. All t-tests were performed using the AFNI command 3dttest++.

Covariance of ASD symptom severity and effects of NFT

We also investigated whether BOLD signal activation changes resulting from NFT covaried with ASD symptom severity and other behavioral and/or demographic information.

Three repeated-measures ANOVAs were performed for the different task conditions and/or activation contrasts. These included one for the imitation task condition only (not the contrast), the imitation contrast, and the combined imitation + action observation contrast. For each 2x2x5 ANOVA, group status (2 levels, ASD or TD) was the between-subject factor, while training status (2 levels, pre or post) and activation cluster (5 levels, one for the average beta value of activation for each cluster showing a significant interaction in the whole-brain ANOVA for each contrast) were within-subjects factors. Covariates included individual scores for pen-and-paper and interview-based diagnostic assessments, age at the beginning of training, the total hours of NFT completed by each individual, and the density of training (defined as the hours of training divided by the number of months within which each participant's training was completed). All ANOVAs were Bonferroni corrected at a significance level of $p = 0.05$.

Additionally, since only the ASD group had scores for ADOS and ADI, a separate set of 3 repeated measures ANOVAs that included these scores was performed for the ASD group only. These ANOVAs included training status and BOLD activation for each cluster as within-subjects factors, no between-subjects factors, and the same covariates as before with the addition of total ADOS and ADI scores.

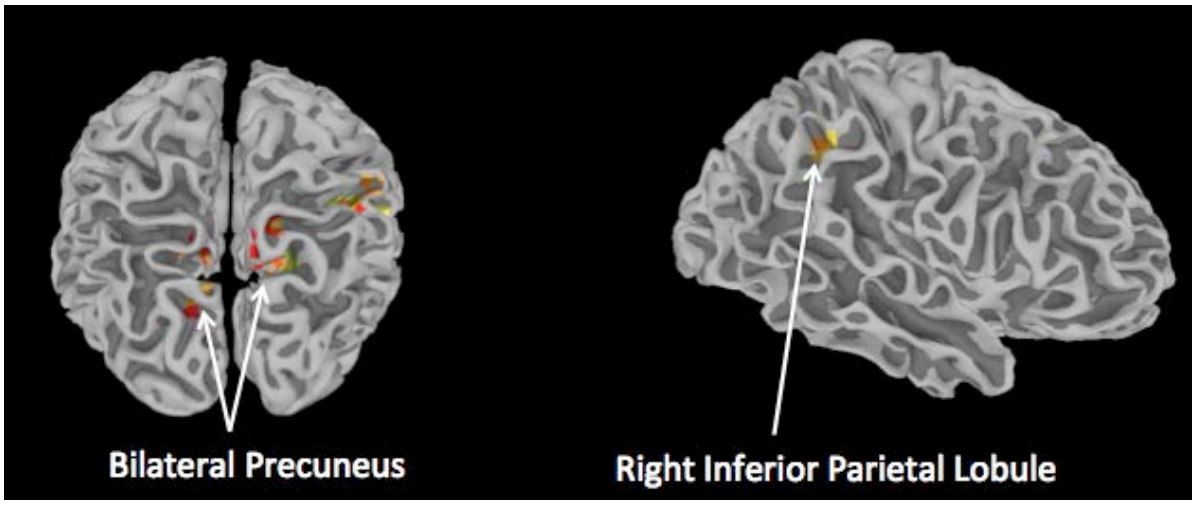
Results

Brain Activation

A two-factor ANOVA was performed for each of three task activation contrasts. These contrasts described in detail earlier, included 1) imitation, 2) action observation, and 3) combined imitation plus action observation. Results reported below for these ANOVAs survived FDR-based correction for multiple comparisons, and all of the clusters reported have a corrected p-value of 0.05 or less.

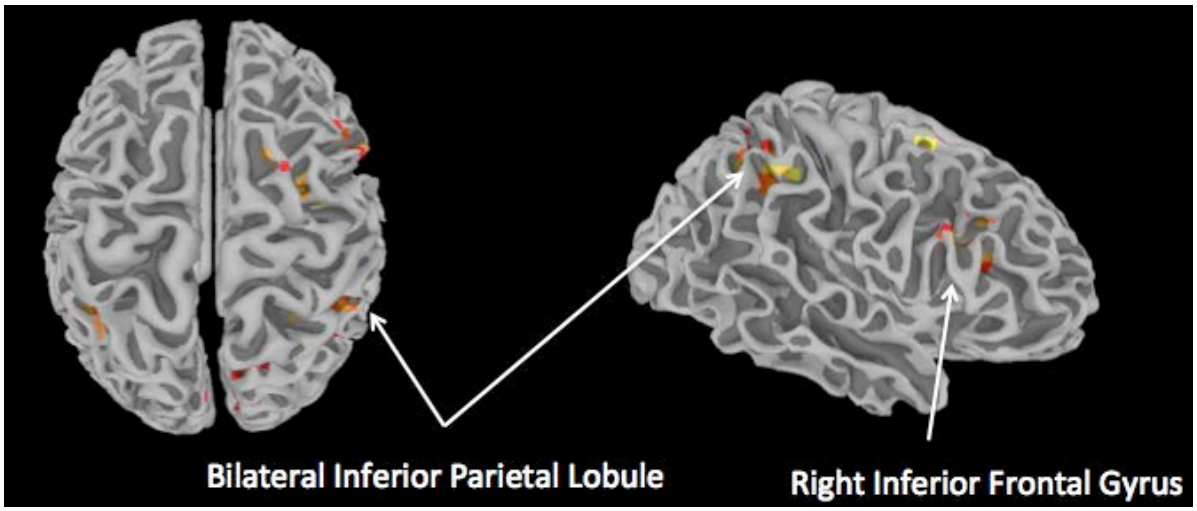
Five significant clusters of interaction between group status and training status were observed for the imitation contrast. These were located in right precuneus, left precuneus, anterior cingulate cortex, right inferior parietal lobule, and left middle frontal gyrus. The locations and sizes of these clusters are listed in Figure

Table 2A: 2 Factor ANOVA (group, timepoint) for Imitation Contrast							
			Talairach coordinates				
Main effects:	Peak Location	Hemisphere	x	y	z	Volume (voxels)	F-statistic
Group x Timepoint Interactions	Precuneus	R	-7	75	46	72	7.63
	Precuneus	L	10	78	39	64	6.54
	ACC	L & R (midline)	0	-24	32	53	4.64
	IPL	R	-41	54	52	49	3.71
	Middle Frontal Gyrus	L	37	-48	25	41	6.23



Five significant clusters of interaction between group status and training status were observed for the combined imitation plus action observation contrast. These were located in right inferior parietal lobule, right precuneus, right inferior frontal gyrus, left inferior parietal lobule, and right superior frontal gyrus near the frontal eye field. The locations and sizes of these clusters are listed in Figure \ref{fig:anova59table} and shown in Figure

Table 2B: 2 Factor ANOVA (group, timepoint) for Combined Imitation + Action Observation Contrast							
			Talairach coordinates				
Main effects:	Peak Location	Hemisphere	x	y	z	Volume (voxels)	F-statistic
Group x Timepoint Interactions	IPL	R	-37	54	46	107	4.40
	Precuneus	R	-14	78	42	91	11.12
	IFG	R	-54	-14	32	53	5.82
	IPL	L	31	47	36	48	4.75
	SFG (FEF?)	R	-24	-7	59	39	5.39



No significant interactions or main effects were observed for the task contrast of action observation alone.

Average beta values of activation for each significant cluster of interaction found in the ANOVAs for each activation contrast were extracted from each participant's data. These beta values were used in a repeated-measures multivariate ANOVA, along with several covariates based on diagnostic and demographic data. The average beta values for these clusters, as well as demographic data used in the covariate analysis, are summarized in Table X.X.

The locations and t-scores of clusters of peak activation (based on between- and within-group t-tests) for each group are summarized in Table 3A and figure x.x (for the imitation contrast) and Table 3B and figure x.x (for the combined imitation + action observation contrast). The locations and t-scores of significant group differences from these t-tests are listed in Table 4A and figure x.x (for the imitation contrast) and Table 4B and figure x.x (for the combined imitation + action observation contrast).

Behavioral and Motion Data

Groupwise accuracy and reaction time data during the imitation task is summarized in Figure \ref{fig:taskbehav}. Group averages for root mean squared difference (RMSD) of head motion during the task scans are summarized in Figure \ref{fig:rmsd}. Overall, differences in task accuracy and head

motion during task scans were negligible, both between-groups and between training status (pre versus post). There was a significant difference in reaction time between groups, with the ASD group having a slower reaction time. This between-group difference was statistically identical before and after neurofeedback training.

Table 5A. Task performance				
	ASD	TD	t-value	p<
Pre-training % Correct trials	79.2%	86.6%	1.1715	0.2641
Pre-training Reaction time (correct trials)	1.20 s	1.14 s	1.9681	.0493*
Post-training % Correct trials	90.7%	89.1%	0.1283	0.9001
Post-training Reaction time (correct trials)	1.19 s	1.04	4.0859	.00005*

Table 5B. Task RMSD Motion				
	ASD	TD	t-score	p<
Total motion RMSD (pre)	0.2575	0.3777	1.3873	0.1691
Percent censored (pre)	0.885714286	0.86	0.3460	0.7353
Total motion RMSD (post)	0.2356	0.2295	0.1173	0.9069
Percent censored (post)	0.98	0.991428571	0.8561	0.4087

Pen-Paper and Parental Assessments

Between-group t-tests were performed on the data from each behavioral and parental assessment collected. Group averages, as well as the results of these statistical tests are summarized in Figure \ref{fig:demopre} (pre-training). Groups did not differ significantly in age and IQ (as measured by the WASI), but the ASD group scored significantly higher (more socially dysfunctional) on the SRS.

Covariance of ASD symptom severity and effects of NFT

Main effects and interactions observed in the series of repeated-measures ANOVAs are summarized in Table X.X.

For the imitation condition activation only (not the full contrast), there was a significant interaction of clusters by group ($p = 0.044$), and of training by clusters by density of training (hours per month) ($p = 0.046$).

For the imitation contrast, there was a main effect of training ($p = 0.025$), as well as significant interactions of training by WASI full score ($p = 0.035$), training by total SRS score change ($p = 0.007$), and training by group ($p = 0.028$).

For the imitation + action observation contrast, there was a significant interaction of training by group (p = 0.047), cluster by age (p = 0.022), and training by cluster by group (p = 0.029).

For ASD only tests:
Ados multivariate imitation condition (not contrast): cluster * ados_scplusrep (p = 0.041)
This interaction appeared to be driven by …
No within-subjects effects for ados

Atecchange

For the imitation condition activation only (not the full contrast), there was a significant interaction of:
Multivariate tests: cluster * atecchange (p = 0.016)
This interaction appeared to be driven by …
No within-subjects effects for atec_change

Nothing for srs_total_change for any condition

Preage: imitation condition (not contrast): multivariate tests: training * cluster * preage: (p = 0.041)

Discussion

The present study investigated the effects of 20-30 hours of mu-based neurofeedback training on imitation-related brain activation and social behaviors in a group of children and adolescents with ASD and a matched group of typically developing (TD) children. We hypothesized that mu-NFT would have positive behavioral benefits for children with ASD since mu rhythms behave abnormally in ASD when compared to TD. We further hypothesized that behavioral changes would be accompanied by neurophysiological changes following the training; specifically, increased activation in areas of the human mirror neuron system. We predicted that both behavioral improvements and neurophysiological changes would be larger in individuals with more severe symptoms of ASD.

In a 3-factor ANOVA examining brain activation during an action imitation and observation task, with activation magnitude, group status (ASD vs. TD) and training status (post and pre) as factors, we found statistically significant interaction effects of training with group status. These effects were driven both by post-training increases in activation in the ASD group along with post-training decreases in activation in the TD group. When the interaction effects were unpacked with t-tests, both the ASD group increases and TD group decreases were found to be statistically significant. The improvements in activation in the ASD group were accompanied by positive changes in behavioral assessments, making it seem probable that the increased activation was the result of more efficient recruitment of areas involved in imitation.

Interestingly, the increases in functional activations seen after neurofeedback training in the ASD group are not seen in the TD group. On the contrary, at the same statistical threshold, a comparison of the post-training versus pre-training TD group activation during both the imitation as well as the imitation + action observation contrasts revealed activations that were lower after the training.

This finding, coupled with negligible changes in behavioral assessments in the TD group, stands in contrast to the apparent positive effects on the ASD group, and demands a more nuanced interpretation of the effects of NFT. One possibility is that in a normally functioning brain, NFT may have the effect of increasing efficiency of network function, resulting in less metabolic activity when the mirror network is activated. Likewise, in brains of individuals with ASD, NFT may increase network connectivity and allow previously neglected areas to be utilized during functional activations, thus increasing the metabolic activity detected as BOLD signal in those areas. Another factor that may have led to this result is the difference in total training time completed by the TD group compared to the ASD

group. There was a statistically significant difference in both total training time and training density (defined as hours of training per month), with the TD group having an average of 17.2 hours compared to 25.5 hours in the ASD group. The TD group had a training density of 1.94 HPM, while the ASD group had 3.75 HPM.

Clusters of significant interaction in the imitation only condition (not the contrast) included one in the right frontal eye field, anterior cingulate cortex along the midline, right inferior frontal gyrus pars opercularis, left precentral gyrus, and left middle prefrontal gyrus.

Clusters of significant interaction between group status and training status for the imitation contrast were located in right and left precuneus, anterior cingulate cortex along the midline, right inferior parietal lobule, and left middle prefrontal gyrus.

Each of these clusters are found in areas important for a wide variety of tasks, and suggest a Another cluster in which there was a significant interaction of group status and training status in the imitation contrast was in the bilateral inferior parietal lobule (IPL). IPL plays a significant role in sensorimotor integration and is one of the defining areas of the core human mirror neuron system. It receives input from the visual system via the superior temporal sulcus, and feeds this input into the ventral premotor cortex (particularly the IFG). IPL has shown hypoactivation in previous studies of action observation and imitation in ASD. Prior to the training, the ASD group did not show statistically significant activation in IPL, whereas the TD group showed some. In direct between-group t-tests this difference was not statistically significant, but the within-group results give some indication that recruitment of this important area during the task was compromised in the ASD group. The fact that activation increased here for the ASD group following the training indicates that NFT may have improved the connectivity of the mirror network resulting in greater recruitment of its constituent areas, though the exact mechanism of this change is not clear.

The largest cluster of significant interaction between group and training for the combined imitation and action observation contrast was located in right inferior parietal lobule, near the angular gyrus. There was also a smaller cluster in the same region in the left hemisphere.

Another important cluster of interaction in the combined contrast was located in the inferior frontal gyrus (IFG).

Other clusters of interaction in the combined contrast included one in right precuneus and one in right superior frontal gyrus, possibly in the frontal eye field.

No significant interactions were found for the action observation only contrast. This could have been the result of insufficient statistical power, since this contrast was expected to show similar albeit less pronounced effects as the others.

Not only does the present study demonstrate changes in imitation-related brain activation resulting from NFT, it also shows a covariance of these neurophysiological changes with positive changes in assessments of social and adaptive behaviors. One interaction was found between training status and total SRS scores, with both groups improving to some degree in their scores. This result suggests that NFT can lead to positive behavioral benefits regardless of the trainee's neurophysiological status. A three-way interaction was found between training status, MNS activation in the imitation contrast, and difference in ATEC score following the training. When unpacked using t-tests, this interaction was driven by increases in MNS activation, as well as increases in ATEC scores, in the ASD group after the training. This suggests that increases in MNS activation resulting from the training were accompanied by positive changes in parental assessments of behavior.

We did not, however, find an interaction between pre-training ADOS scores and the magnitude of functional activation changes following the training. If anything, the trend seems to be that

Our findings have implications for operationalizing the benefits of NFT towards practical solutions to the early diagnosis and possible repair of hMNS deficits in autism. While data collection for this study is ongoing, these preliminary results provide evidence that mu-NFT has significant positive effects on social behavior, as well as on the neuroanatomical substrates underlying that behavior, in

children and adolescents with ASD. Future studies involving larger sample sizes and sham-training control groups may increase the impetus for use of NFT in clinical settings, as well as for deeper investigations into the mechanisms and applications of NFT. The brain areas in which interaction effects were found in this study, such as anterior cingulate and insular cortices, have been implicated in a wide variety of behaviors. While this study demonstrates that NFT is associated with changes in imitation-related brain activity, future studies may demonstrate the potential of NFT to induce changes in a broad range of neurofunctional domains.

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5. Book chapter

Neurofeedback Training Produces Normalization in Behavioral and Electrophysiological Measures of High Functioning Autism

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Abstract:

Autism Spectrum Disorder (ASD) is a neurodevelopmental condition typified by impairments in behavior, social, and communication skills. Recent observations have shown that the autistic brain exhibits aberrant functional connectivity that can impact synchronization and effective neural communication. This can lead to atypical sensorimotor processing critical for normal social cognition. We tested the efficacy of neurofeedback training (NFT) in reducing symptoms in children with high-functioning ASD by targeting training to the mirror neuron system via modulation of EEG mu rhythm. The effects of 30 hours of NFT on ASD and on typically developing (TD) children were assessed using standard and blind source separation techniques. ASD and TD subjects completed an eyes open/closed EEG session as well as a mu suppression index assessment before and after training. Additionally, parents filled out pre and post behavioral questionnaires. The results show behavioral and electrophysiological improvements in ASD subjects following NFT, including changes in mu suppression. The effects on TD subjects appear to be different from those in children with ASD. This suggests that while induction of neuroplastic changes via NFT can produce normalization in dysfunctional mirroring networks in children with autism, the benefits do not extend to typically developing brains.

Keywords: Mirror neuron system; sensorimotor systems; EEG mu rhythms; mu suppression index

Introduction

Autism is currently one of the most researched areas in neuroscience. It is a complex neurodevelopmental disorder that impairs a child's development of language, behavior, social, and communication skills [1-3]. There is no template for what characterizes a 'typical' individual with autism since symptoms can range from mild to severe. Low functioning individuals may have problems with speech production while high functioning individuals may have normal IQ levels yet exhibit social interaction deficits. This wide spectrum of symptoms is more commonly known as Autism Spectrum Disorder (ASD).

Currently, ASD is of serious concern because no cure exists and worldwide prevalence has been increasing rapidly in the last few decades. Although little agreement exists as to the exact causes for this increased prevalence, scientists believe that ASD might be related to a variety of factors, including genetic disorders, mitochondrial disorders, environmental factors, or atypical brain development [4-6]. An interesting observation is that generally males are 4-5 times more prone for developing autism than females. An autism-risk gene, CACNA1G, has been found that is more common in males than females, suggesting that it might be an important clue to the sex differences [7].

One mechanism hypothesized to underlie the social impairments associated with high functioning autism (HFA) is a dysfunctional Mirror Neuron System (MNS) [8-10]. The discovery of mirror neurons in monkeys and an MNS in the human brain has provided a neurological substrate for understanding many key concepts in human social cognition directly relevant to the behavioral and cognitive deficits observed in ASD [11], including the ability to comprehend actions, glean intentions, and learn through imitation. First described by Rizzolatti and colleagues in the macaque monkey[12], mirror neurons are thought to be involved in both self-initiated action and the representation of action performed by others. Neurons in the pars opercularis of the inferior frontal gyrus (IFG) show increased firing while executing *and* observing the same action, representing a potential mechanism for mapping seeing into doing [13,14]. As has been noted in a number of recent reviews, deficits in MNS activity may explain the abnormal social skills prevalent in ASD, such as impairment in joint attention, understanding the intentions of others, and empathy – a condition also referred to as "mind-blindness" [15, 16].

Although some studies have raised questions about the role of mirror neurons in human social behavior [17, 18], an increasing amount of work suggests that a dysfunction in the MNS does contribute to social deficits [9, 19-23]. Specifically, impairments likely arise from an inability to "form and coordinate social representations of self and others via amodal or cross-modal representation processes" [24] – the type of function ascribed to mirror neurons. A particularly relevant fMRI study by Dapretto et al. [25] demonstrated decreased activation in the IFG (pars opercularis) in individuals on the autism spectrum, and activity in this region was found to be inversely related to symptom severity in the social domain. EEG studies have also shown that putative electro-biomarkers of MNS activity exhibit abnormalities in ASD compared to TD children [21, 23, 26, 27]. Nonetheless, despite the excitement generated by these observations, few if any investigations have focused on operationalizing such insights towards practical solutions to the early diagnosis and possible repair of MNS deficits.

Direct recording of neural activity using electromagnetic methods have unveiled activation patterns correlated with mirroring [14, 28, 29]. These scalp-recorded EEG patterns of activity occur in the alpha (8-12 Hz) and beta (15-25 Hz) range, are most evident over the central region of the scalp overlying the sensorimotor cortices, and are modulated by motor activity [30]. Traditionally these EEG patterns have been labeled "*mu rhythms*" (reviewed by [14]) that reach maximal power in the absence of overt movements when an individual is at rest. Mu rhythms are desynchronized and power reduced when a hand or a foot movement is prepared, and almost disappear when the movement is actually performed [14, 31]. They are also affected by cognitive processes such as memory [32-34], selective attention [35, 36] and affect [37-39]. Particularly relevant to this chapter is the evidence that during self-initiation,

observation, and even imagination of action in typically developing (TD) individuals, the MNS network is active and power in the mu rhythm is suppressed [30, 40-42].

Several studies have demonstrated that individuals with ASD exhibit abnormal mu rhythm suppression, suggesting that their mirroring system does not engage normally when observing someone else's movements[8, 26]. As argued above, deficits in MNS activity provide a basis for problems in higher order social cognition such as empathy, theory of mind, imitation and language. If true, then one hypothesized method for recovering MNS function and ameliorating these behavioral deficits is neurofeedback training (NFT), an operant conditioning technique that results in the self-regulation of brain electrical oscillations. As an intervention, NFT has been used primarily in clinical settings and therefore efficacy is based largely on case studies with few large randomized, controlled, and blinded studies. Nonetheless, a substantial amount of work supports the rationale for its use in the context of treatment [43-49]. It is well recognized that more than 50% of ASD individuals demonstrate significant EEG abnormalities [50-52], with upwards of 30% developing clinical seizures by adolescence. Even when clinical seizures have not been identified, more than 50% show paroxysmal sharp discharges, especially during sleep. Additional daytime abnormalities include altered spectral profiles, abnormal patterns of coherence, and reduced mu rhythm activity. These observations have led many clinical practitioners to use EEG-based interventions, such as NFT, as a therapeutic strategy. Supporting this case is strong evidence for the efficacy of this approach towards a variety of other neuropsychological conditions, including ADHD [53-55], epilepsy [56-60], traumatic brain injury [61, 62], anxiety [63], and substance abuse [64].

The main goal of the study described in this chapter was to assess whether NFT provided over a period of many weeks could improve behavior and normalize the electrophysiology in children with HFA. A secondary goal was to assess whether benefits accrue for TD children with no known dysfunctions. In the behavioral component of the study, parents filled out paper and pencil assessments prior to and following NFT. In the electrophysiological component, a 20-channel EEG recording was used to quantify EEG in an eyes open/closed conditions and in a mu suppression index (MSI) task prior to and following NFT. We hypothesized that NFT would normalize abnormal functional connectivity in the ASD brain and that this would be reflected in improved behavioral responses and in normal patterns of electrical activity compared to TD children, who would show similar improvements as the ASD group, or at minimum show no changes.

Research Design and Methods

Participants

A total of 13 ASD (10 males; mean age=11.38 yrs; range=7-17 yrs; stdev=3.86) and 11 TD (7 males; mean age=10.18 yrs; range=8-17 yrs; stdev=2.68) subjects participated. There was gender inequality among the groups due to the fact that more males tend to be diagnosed with ASD compared to females.

Training

Subjects in each of the groups completed approximately 30 hours of neurofeedback training. They came into the lab once or twice a week and completed a session that lasted either 45 or 60 minutes, respectively. Sessions involved three short fifteen-minute video clips plus short rest periods between clips or an hour long DVD. The videos consisted of either cartoon or human based interactions. The DVD was chosen from a variety of children's movies. Subjects had the choice of choosing the videos or DVD they wanted to use at each session. In order for the video clip or DVD to play power in the 8-12 Hz band recorded at the C4 electrode site on the scalp had to be maintained above a pre-determined threshold for at least one second, while theta (4-8 Hz) and beta (13-30 Hz) activity had to remain below pre-determined thresholds. Theta and beta rhythms are typically associated with distraction, unfocused attention and movement. When the theta and beta rhythms exceeded threshold, the video or DVD would pause. To resume playing, the subject had to focus and maintain levels of these frequencies

above (mu rhythm) and below (theta, beta) threshold for at least one second. Thresholds for the three frequencies were determined in an initial baseline period during each session and were calibrated such that performance for the entire session fell in the 75-85% success range.

Electrode Placement

Two clip electrodes were attached to each earlobe, with one electrode acting as reference (right earlobe) and the other as ground, while a third electrode was placed on the C4 site overlying the premotor region of the scalp on the right hemisphere. Thought Technology hardware (ProComp2 bioamplifier; 256 Hz sampling rate) and the Biograph Infiniti software computer program were used to record brain activity and control the NFT sessions.

Behavioral Assessments

Parents filled out three different paper and pencil assessments: the Social Responsiveness Scale (SRS), the Autism Treatment Evaluation Checklist (ATEC), and the Vineland Adaptive Behavior Scales (Vineland-II). The SRS contains sub-categories related to social awareness, social cognition, social communication, social motivation, and autistic mannerisms. Scores $\leq$ 59 are considered within the normal range while scores > 59 are considered to fall in the autism range. The ATEC also contains sub-categories for speech/language/communication, sociability, sensory/cognitive awareness, and health/physical behavior. ATEC scores $\leq$ 5 are considered in the normal range, while > 5 are considered to fall in the autism range. The Vineland Adaptive Scale includes sub-categories for communication, daily living skills, and socialization. Scores of 90 or greater are considered in the normal range while less than 90 are considered in the autism range. Assessments were filled prior to the start of any NFT sessions and following the completion of the 30 hours of training. All thirteen parents of ASD children completed the SRS and ATEC forms, while 12 completed the Vineland. Compliance from TD parents was less consistent with 7 completing the SRS and Vineland, while 9 completed the ATEC.

Electrophysiological Assessments

At the beginning and end of 30 hrs of NFT subjects were prepped with a 20-channel EEG cap (including one channel for the electrooculogram) for the eyes open/closed and MSI assessments. During recording sessions, subjects were asked to first close their eyes for approximately 10 minutes and to remain still avoiding major movements of head and body. After a brief rest period, they were asked to maintain eyes open for another 10 minutes while minimizing body/head movements. For the MSI assessment subjects were asked to watch 2-min biological movement videos of simple (Hand, Crayon, Biomotion) and complex (Social Play) actions or of non-biological movements (Balls), in addition to making self-initiated movements with their right hand when signaled to do so on the screen. The Hand video showed a right hand making a duck movement (bringing the thumb and other fingers together rhythmically every second). The Crayon video showed a right hand taking a crayon out of a crayon box and putting it back into the box rhythmically at least once a second. The Biomotion video involved a point-light display of an adult male jumping rope. The Social Play video displayed three individuals (two females and a male) tossing a small ball around to each other. Lastly, the Self-Initiated Movement involved subjects making the duck hand movement with their right hand as seen in the Hand video.

Blind Source Separation (BSS)

EEG data collected during the eyes open/closed assessment were processed using the EEGLab toolbox [65] for Matlab. Data were bandpassed from 3-40 Hz with an offline FIR filter. Artifacts such as eye blinks, EMG, or noisy channels were visually identified and removed. Data were then re-referenced to the average channel value and sectioned into two-second epochs for averaging in frequency space. Pre- and post-training datasets were concatenated for homogenous source space separation. EEG source estimates were obtained using the Infomax ICA algorithm, and dipole positions estimated with the DIPFIT 2.x toolbox on an MNI Boundary Element head model. Power spectra were averaged across subjects for channels C3, C4 and Cz and then a mu cluster in source space was computed and averaged across subjects. Source estimates were clustered by dipole locations into 10 clusters via K-means. The

cluster was identified as a mu component based on scalp distribution (central, left/right lateralized), dipole estimate (e.g., pre-central gyrus, Talairach: -61, -11, 29) and spectral profile including discernible peaks at 10 and 20 Hz. In the MSI measures, individual differences in power spectra are controlled via ratios of active condition to a control condition. To correct for individual differences during eyes open and eyes closed tasks we computed a ratio dividing the active condition by data from the first 10% of each condition as a baseline period. With the power spectra ratios derived, a repeated measures ANOVA was employed to compare the mu (8-12 Hz) portions of the data in both channel and component space. A section of the low beta (16-22 Hz) was also compared across conditions to observe any possible harmonic effect of the mu activity.

Results

Behavior. SRS, ATEC and Vineland data were analyzed using a repeated measures ANOVA with Treatment (pre, post) and Subcategories as within subject factors and Group (ASD, TD) as a between subject factor. The Greenhouse-Geisser correction for degrees of freedom was used in determining significance while multiple comparisons were Bonferroni corrected.

Social Responsiveness Scale (SRS). The SRS results indicated a main effect of Group, $F(1,12)=109.46$, $p<.001$ and a Treatment x Group interaction, $F(1,18)=5.73$, $p<.05$. As illustrated in Figure 1A, there was an overall significant difference between pre/post ASD scores and pre/post TD scores, with ASD group scores exceeding threshold for an autism diagnosis, while TD scores fell below the threshold. Separate one-way ANOVAs showed a main effect of Treatment, $F(1,12)=10.56$, $p<.01$ and a main effect of Subcategories, $F(4,48)=6.76$, $p < .001$ for the ASD group. The TD group did not show a Treatment effect but only a main effect of Subcategories, $F(4,24)=5.32$, $p<.01$. The significant decreases in scores as a function of treatment support the hypothesis that children with autism improved their behavior following NFT.

**** Insert Fig. 1 about here ****

Autism Treatment Evaluation Checklist (ATEC). As shown in Figure 1B, analysis of the ATEC scores disclosed a main effect of Group, $F(1,20)=96.29$, $p<.001$, indicating that pre- and post-training ATEC scores were significantly higher for the ASD than TD group. Mean ASD scores (8) exceeded the threshold for autism (5), while TD scores (1.6) remained below that threshold. However, there was a significant Treatment x Group interaction, $F(1,20)=24.55$, $p<.001$, indicating that treatment improved ASD scores by lowering them to 6.2, while TD scores increased to 3.0, although still below the normal cutoff point. The results also showed a main effect of Subcategories, $F(3,60)= 29.17$, $p<.001$ and specifically a Group x Subcategories interaction, $F(3,60)= 11.62$, $p<.001$. Analysis of that interaction indicated that overall higher ATEC scores occurred for all subcategories in the ASD compared to TD group. Finally a 3-way interaction of Group x Treatment x Subcategories, $F(3,60)= 6.01$, $p<.01$ indicated that while ASD scores in all subcategories were reduced post-training, they increased for the TD group. Individual one-way ANOVAs for the ASD and the TD groups showed significant Treatment effects for both groups, ASD: $F(1,12)=14.01$, $p<.01$, TD: $F(1,8)=15.10$, $p=<.01$. The significant decrease in ATEC scores between pre- and post-training support the hypothesis that NFT improved ASD behavior, although TD scores were changed in the opposite direction.

Vineland Adaptive Behavior Scales. As illustrated in Figure 2, there was a significant main effect of Group, $F(1,17)= 36.43$, $p<.001$, with TD subjects showing normal scores (above the threshold), and ASD subjects showing scores below the threshold. A marginally significant Treatment x Group interaction, $F(1,17)=4.20$, $p=.056$ followed by two separate one-way ANOVAs showed that there were no significant effects in the ASD group, while the TD group showed a marginally significant score increase, $F(2,12)=3.72$, $p=.055$, indicating a slight regression in scores for these subjects.

**** Insert Fig. 2 about here ****

Standard Electrophysiology

Nineteen EEG electrodes were divided into five different electrode clusters for statistical analysis: frontal (F7, F8, F3, F4, FP1, and FP2); centro-parietal (C3, C4, P3, and P4); temporal (T3, T4, T5, and T6); occipital (O1 and O2); and midline (Fz, Cz, and Pz). For each cleaned segment of EEG, the integrated power in the 8–12 Hz range was computed using a Fast Fourier Transform. Data were segmented into epochs of 2 s beginning at the start of the segment. Fast Fourier Transforms were performed on the epoched data (1024 points). A cosine window was used to control for artifacts resulting from data splicing. Mu suppression was computed by taking the log base 10 of the mu power in the 8-12 Hz band during the experimental condition divided by the baseline (Ball) mu power. A repeated measures ANOVA was used to analyze mu suppression for the different clusters using Video (Hand, Crayon, Biomotion, Social Play and Self-Initiated Movement), Electrodes, and Treatment (pre, post) as within subject factors, and Group (ASD, TD) as a between subject factor.

There were no main or interactive effects in the Frontal or Temporal electrode clusters. The Occipital cluster showed a main effect of Video, $F(4,36)=4.11$, $p<0.05$, indicating that suppression effects occurred only in the Social Play condition
(-.105). However, no other main or interactive effects were noted at occipital sites. Indeed, the primary set of electrodes showing effects were in the centro-parietal and midline clusters.

Centro-Parietal Cluster: As shown in Fig. 3, a main effect of Video, $F(4,36)= 6.69$, $p < 0.01$ indicated that mu suppression in this cluster was largest for Self-Initiated Movement (-.200) followed by Social Play (-.107) and then Hand (-.082) movement. A main effect of Electrodes, $F(3,27)=3.76$, $p<0.05$ disclosed larger mu suppression at central (C3, C4) compared to parietal (P3, P4) sites and larger over right (C4: -.205) compared to left (C3: -0.100) sites. A Video x Electrodes effect, $F(12,108)=3.31$, $p < 0.05$ indicated that for all video conditions, except Biomotion, there was overall larger mu suppression on the right (C4) compared to left (C3) sites. Finally, as illustrated in Fig. 4A, a Treatment x Electrodes interaction, $F(3,27)=3.79$, $p=0.052$ indicated that training produced larger mu suppression at most sites (C3, P3, P4) but reduced it at the C4 sites.

Midline Cluster. Analysis of midline electrodes showed no main effect of Group or Treatment but a marginally significant Treatment x Group interaction, $F(1,9)=4.68$, $p=.059$, indicating that while mu suppression in the ASD group became more negative from pre- to post-training (.003 vs. -.029), it became more positive for the TD group
(-.040 vs. .088). Furthermore, as shown in Fig. 4B, a marginally significant Treatment x Group x Electrode, $F(2,18)=3.87$, $p=0.052$ showed that the enhanced mu suppression in the ASD group and the decrease in TD group centered on the fronto-central sites.

**** Insert Figs. 3 and 4 about here ****

Quantitative EEG

Separate multivariate tests were carried out for mu and beta frequency bands in both channel and source space. For channel space, a repeated measures ANOVA was computed using Task (eyes open, eyes closed), Treatment (pre-, post-training) and Electrode (C3,Cz,C4) as within subject factors and Group (ASD, TD) as between subject factors. The source space tests used the same ANOVA factors, with the exception of Electrode, as this was only carried out on one localized cluster of activity. Multiple comparisons were controlled with a Bonferroni correction.

Channel Space. At the central electrodes there was a main effect of Task in the mu band ($F(1,7)=6.34$, $p<0.05$). This indicates a smaller baseline corrected value for the eyes closed task than the eyes open task. This likely indicates that the ratio of activity between the baseline window and active portion of the QEEG session were more similar for eyes closed than for eyes open. There was also an

interaction between Task x Electrode ($F(2,6)=6.71$, $p<0.05$). As shown in Fig. 5A, for the eyes open task, Cz and C4 exhibit a similar power, which is significantly smaller than mu power at C3. For the eyes closed task, C3 resembles Cz, both of which are lower power than at C4. A three way interaction between Treatment x Electrode x Group ($F(2,6)=6.98$, $p<0.05$) suggests different changes between groups at different electrodes (see Fig. 5B). ASD subjects exhibited an increase in power at site Cz from the pre-training session to the post-training assessment. TD subjects exhibited increases at all central electrode as a result of NFT, with greatest increases exhibited at C3. In the beta band a marginally significant effect was observed for task ($F(1,7)=5.51$, $p=0.051$), and Group x Treatment interaction, ($F(1,7)=3.75$, $p=0.094$). Larger beta power was observed for ratios eyes open condition rather than eyes closed. TDs showed an increase in Beta after NFT training.

**** Insert Fig. 5 about here ****

Source Space. A mu cluster centered on the left pre-central gyrus (Talaraich -61, -11, 29) was identified, as illustrated in Fig. 6A. There was no main effect of participant group in source space, but there was a significant interaction between Treatment x Task x Group, ($F(1,12)=6.35$, $p<0.05$). There was little change in the eyes open task between pre and post training assessments in either subject group. However, there is a divergence in direction of change between ASD and TDs for the eyes closed task. TD participants increased mu power from pre-training to post-training (1.001 to 1.020) whereas ASDs decreased mu power, and by a larger factor than TDs (1.021 to 0.987). No significant effects or interactions were observed for the beta band component.

**** Insert Fig. 6 about here ****

Discussion

The results from this study are consistent with the mirror neuron theory of autism, which proposes that the varied social dysfunctions found in autism spectrum disorder (ASD) can be accounted for by a dysfunctional mirror neuron system (MNS) [9, 11, 23, 66-68]. ASD is a neurodevelopmental condition that impairs a child's maturity in terms of communication, motor, and/or social skills. Children with ASD, and in particular those with high functioning autism, exhibit problems socializing and understanding the actions and intentions of others, what Baron-Cohen called "mindblindness" [69]. The MNS is a network of brain areas centered in the inferior frontal gyrus and inferior parietal lobes that is activated when individuals observe or perform a goal directed action [13, 70-72]. In humans, this network is assumed to be critical for social cognition, from imitation learning, to theory of mind and empathy[66, 72], aspects of what David Siegel has called "mindsight" [73]. The relationship between mindsight and mindblindness appears to depend on the integrity of the MNS. In our data, there is support for the hypothesis that affecting the modulation of the MNS, as indexed by EEG mu rhythms, can result in behavioral and electrophysiological changes in children with ASD. Furthermore, that neurofeedback training focused on EEG mu rhythms is an effective methodology for gaining control of that modulation.

Children with ASD in the present study exhibited deficits in social cognition and in the suppression of EEG mu rhythms compared to typically developing (TD) controls. This is consistent with previous studies showing a similar pattern of responses [8, 68]. Following 30 hrs of neurofeedback training (NFT), the pre-post changes in behavior and electrophysiology indicated that positive changes occurred in the children with ASD but that those benefits did not translate in a similar way to TD children. The efficacy of NFT as an intervention for autism is still an unsettled question but a variety of studies have shown consistent positive effects [45, 74]. Whether the effects are similar for a normal brain in a typically developing individual is also an unresolved question.

In the present study, the set of validated measures used to assess behavior in both groups included the Social Responsiveness Scale, the Autism Treatment Evaluation Checklist, and the Vineland Adaptive Behavior Scales. These scales encompass subcategories related to sensory/social/cognitive awareness, social cognition/ sociability/socialization, speech/language/social communication, social motivation, and autistic mannerisms, and health/physical behavior, including daily living skills. All these measures improved in the ASD group but showed an opposite trend in the TD group following NFT.

In terms of the electrophysiology, there were no group differences in EEG mu suppression prior to training and indeed both groups showed some expected similarities. First, the foci for mu rhythms and the measured changes were primarily over sensorimotor regions of the brain, proposed to be the source(s) of such rhythms [75-79]. Second, mu suppression responsiveness to movement of increasing complexity showed a gradient, consistent with previous reports [80]. That is, power in the 8-12 Hz band exhibited the largest suppression during self-initiated movement or the execution of movement compared to the observation of movement. No differences were seen whether the observed movement was simple (hand movement) or complex (social play). Finally, larger mu suppression occurred on the right (hemisphere) central site (C4) compared to the left site (C3). All these features argue for significant similarities in mu rhythm neural sources and functional properties in the ASD and TD groups. Nonetheless, quantitative EEG analysis indicated that one main difference between groups in terms of channel space might simply be overall reduced mu and beta power in the ASD compared to the TD group. Furthermore, NFT increased synchronization of mu and beta power in the ASD group but reduced mu power in the TD group. An increased in synchronization occurred during the eyes closed condition in the ASD group.

Additionally, the effects of NFT were to increase mu suppression primarily in the centro-parietal and midline electrode clusters. Within the centro-parietal cluster training enhanced mu suppression at C3, P3, and P4 but reduced it at C4. Along midline sites, the effects were to enhance mu suppression mainly at frontal and central sites and decrease it at parietal sites. Overall, these results indicate that NFT is an effective form of intervention that affects the electrophysiology in specific brain regions,

namely those associated with MNS, and its outcome is behavioral improvements in the social behavior of children with HFA. In contrast, in a normal brain, this type of intervention does not translate into benefits and in fact produces overall reduced synchronization of mu rhythms that leads to decreased social behaviors.

Several methodological limitations add caution to these interpretations. First, the size of the subject pool was relatively small. Long-term and resource intensive studies such as these are difficult to do and accrual of a very large subject population is difficult. For parents and children, the visit to a lab twice a week for 40 sessions requires significant commitment and patience. Although over the years we have learned to limit the number of dropouts and to reduce non-compliance, this is only a small part of the problem. Another limitation is that in order to maintain attentiveness and motivation we allow every child to customize the videos and DVDs they watch during training. It may be that certain videos and movies activate the brain's social networks more than others and we did not control for this. An additional limitation is that autism is primarily a male disorder and hence our subject pool comprised mainly males. Such gender disparity can be overcome with an intense search for female subjects, but that is dependent on time and resources, which were not available for this study. A potential concern in the behavioral component of the study is that parents fill out assessments with high expectations of positive results, especially parents of the children with ASD. Since this is an unblinded study, it may explain some of the results. However, the fact that the electrophysiology is also congruent with the behavioral findings adds support to the idea that these effects are real and not necessarily placebo effects. Somewhat related is that the behavioral scales used have been validated for an autism population and not TD children.

Source space analyses for the electrophysiological data are a new contribution to the literature on the effects of NFT for ASD therapy. We observed some significant effects of treatment for different subject groups in the mu band, but these should be interpreted with caution. The existing QEEG databases are built upon 21 channel EEG recordings. To maintain consistency with the clinical database we used the same 10-20 electrode montage. However, in terms of source space, this is a sub-optimal array, as 64 channels provides significantly more accurate estimations [81] and increasing to 128 channels can yield single dipole estimates approaching 4 mm accuracy [82]. One interesting question that remains with regards to neurofeedback is how training at one electrode site influences activity at other sites. For instance we observed significant changes at the C3 electrode when C4 was the target of training. A common mu source driving both of these electrodes could explain these observations.

With regards to the QEEG, the changes in power, particularly for source space are counter to our predictions. We observed a decrease in mu power for ASD and an increase for TD at the mu cluster. It is important to keep in mind the baseline correction ratio we employed. The farther a score is away from 1, the greater the differences between the first 10% of a trial used as a baseline period and the latter 90% of the trial used as the active period. The eyes closed task, surprisingly, exhibits less coherence between the baseline and active window. One might expect to see greater alpha or mu power throughout the duration of the eyes closed task, which might yield a ratio closer to 1. The eyes open task on the other hand exhibits a more uniform mu power across the trial with ratios for both groups approaching 1. While superficially the decrease in mu power for ASD & increase for TD as a result of training might seem antithetical to the divergence of the two populations in other electrophysiological measures, this may actually corroborate these findings. The direction of divergence is inversely correlated between the electrophysiology measures. The ambiguity between changes at different electrodes and diverging direction of responses further corroborate the need to examine physiological effects of NFT in EEG source space.

Although the results are consistent with the mirror neuron theory of autism, they demonstrate that this type of NFT affects ASD and TD groups differently. The ASD group showed increased or normalized mu suppression over centro-parietal and frontal electrodes following NFT suggesting greater

engagement of the MNS. In contrast, the TD group showed reductions in mu suppression and therefore decreases in MNS engagement. More research is needed to tease these effects apart.

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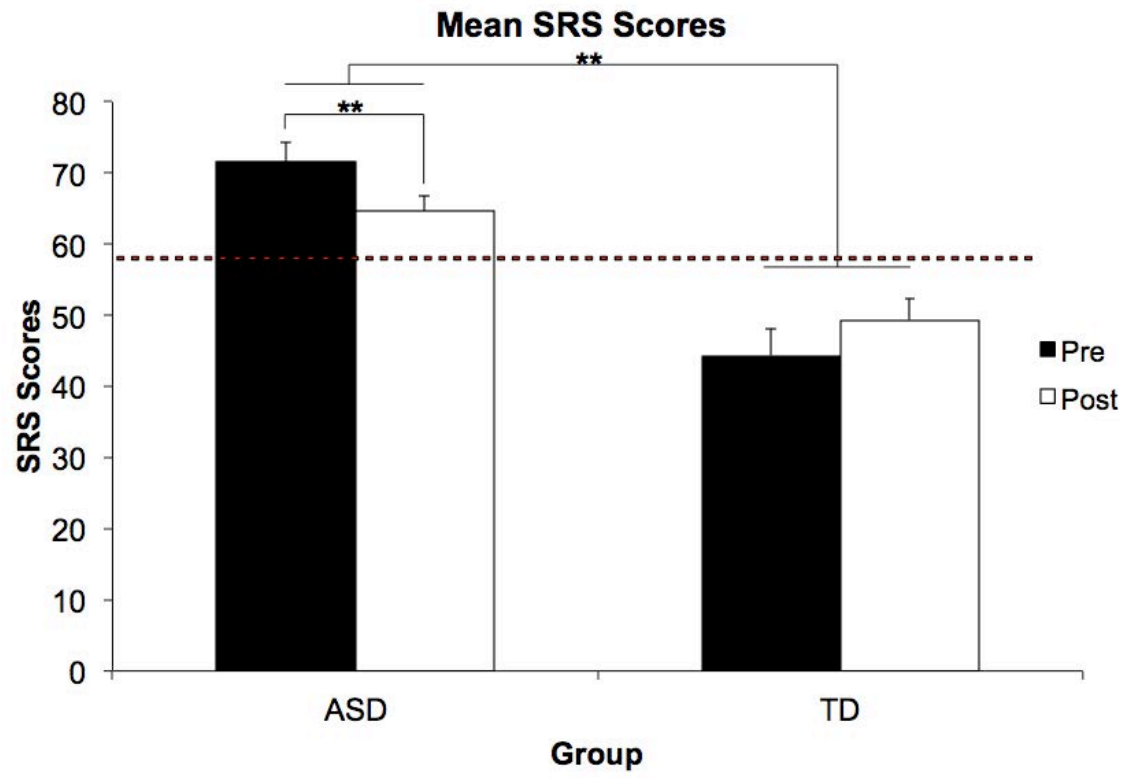
Figures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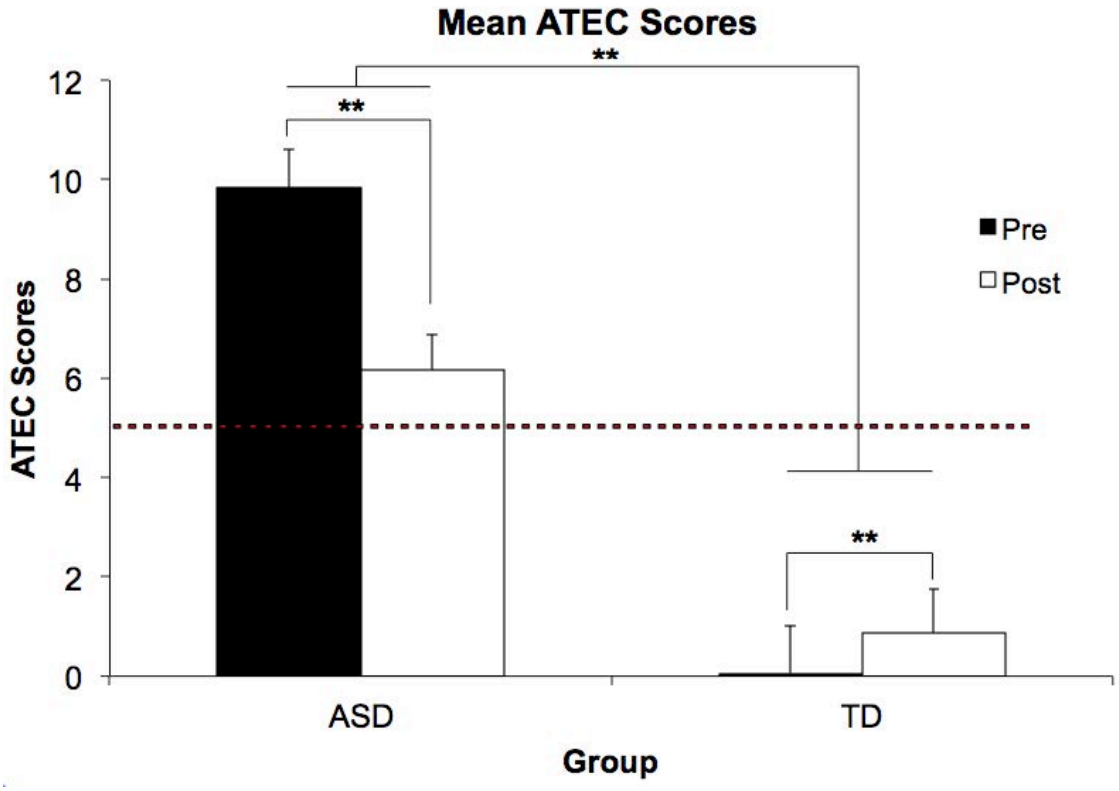
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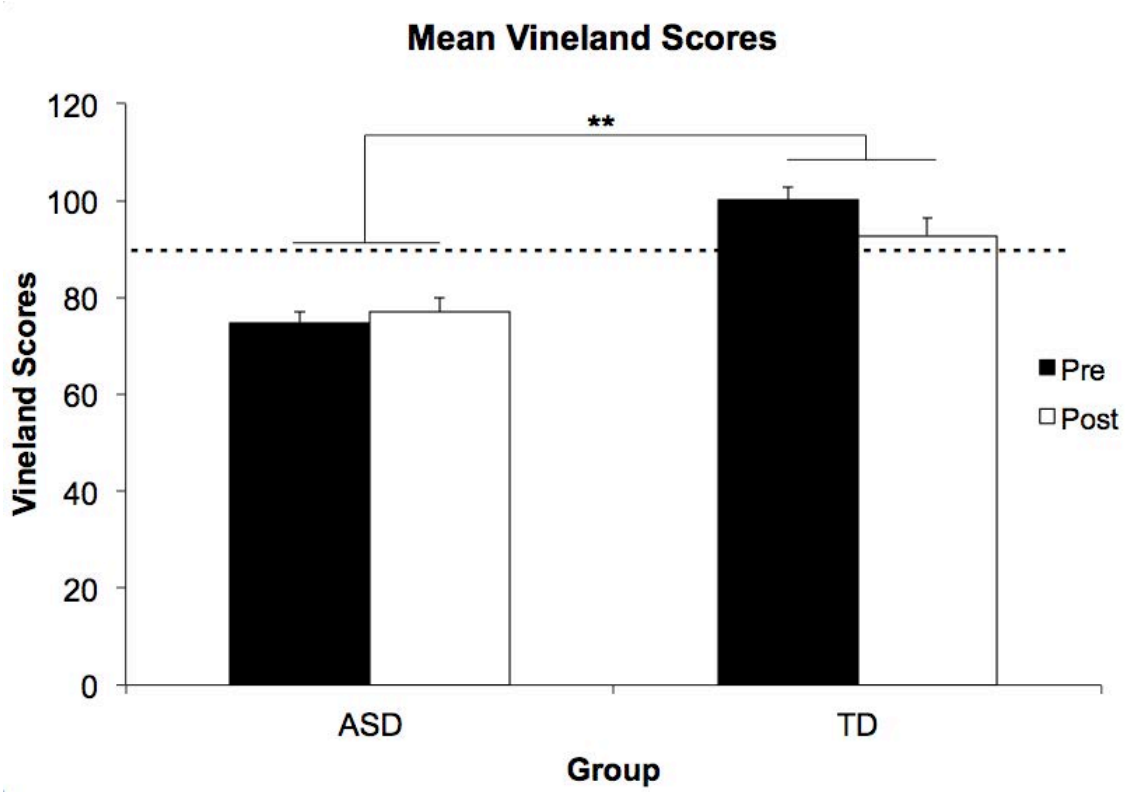
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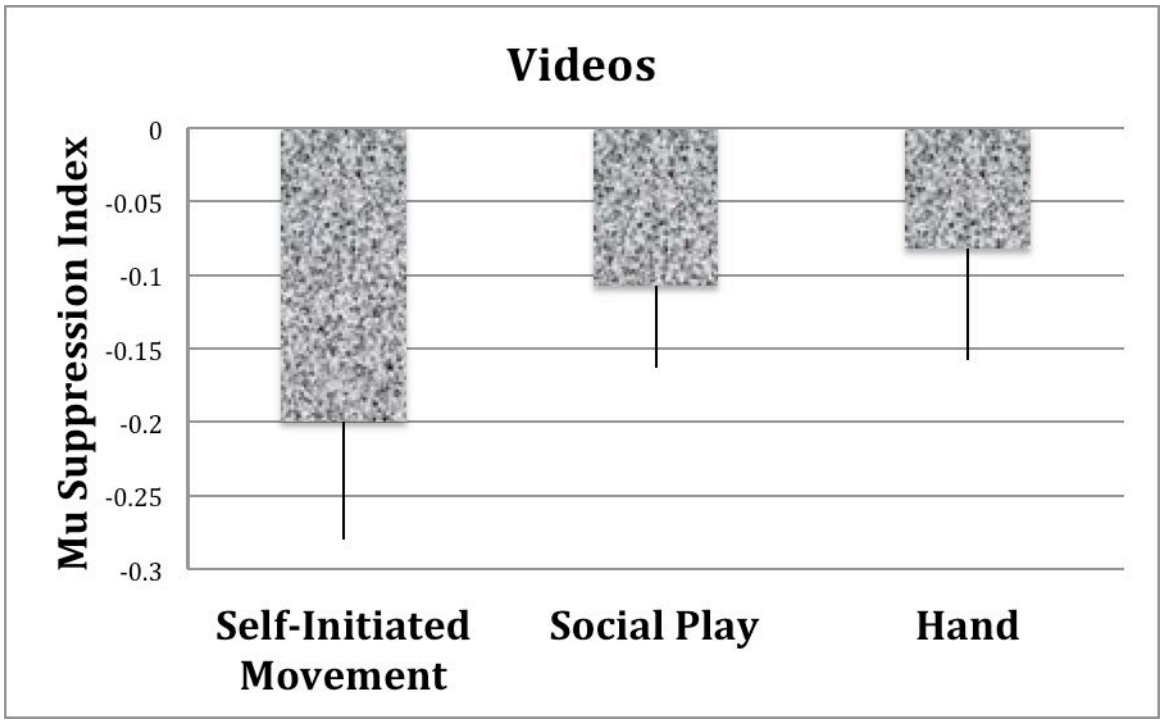
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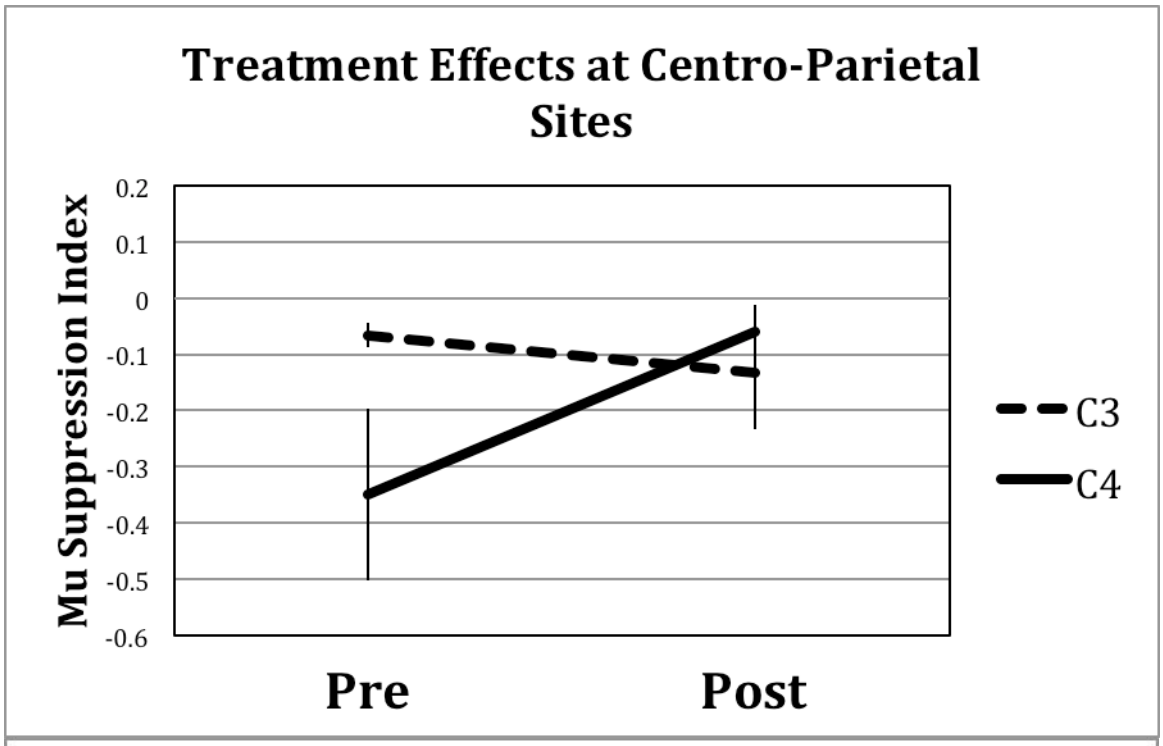

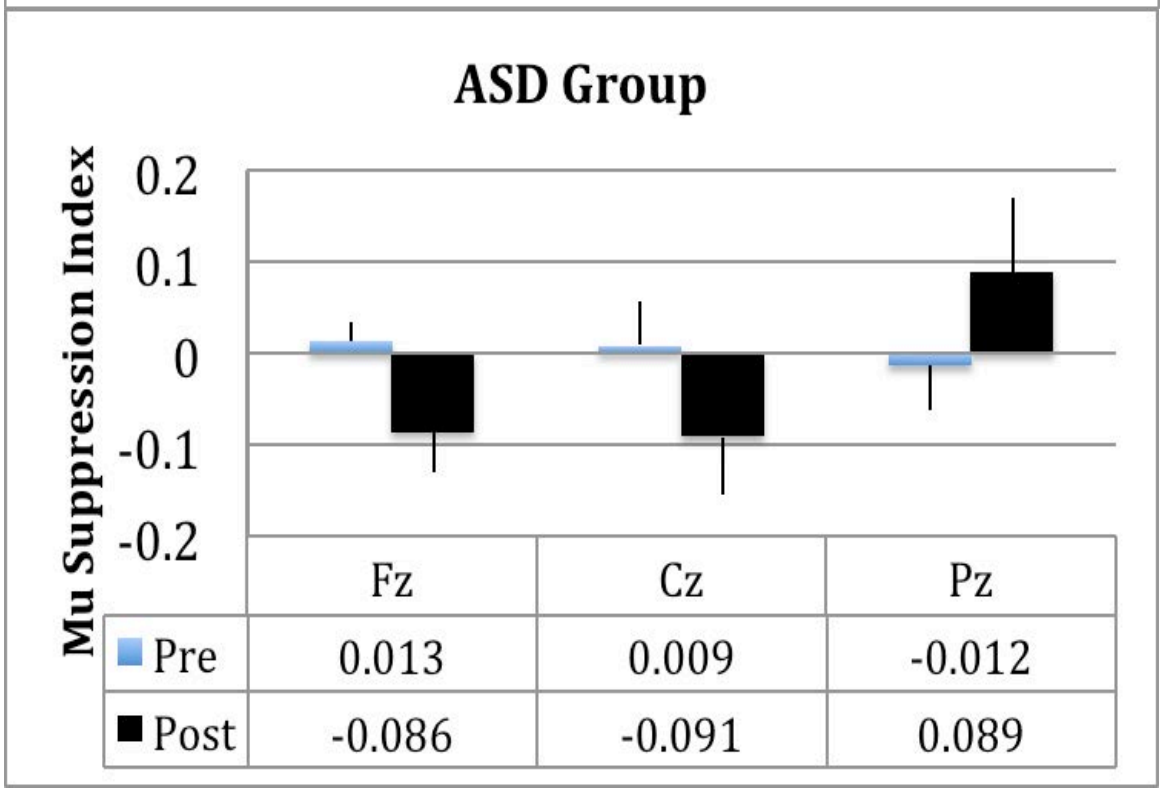

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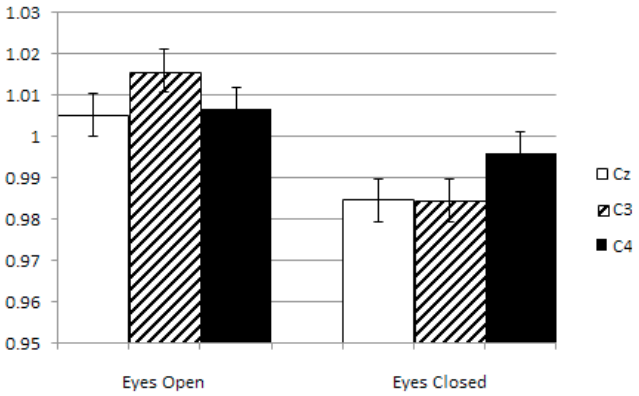
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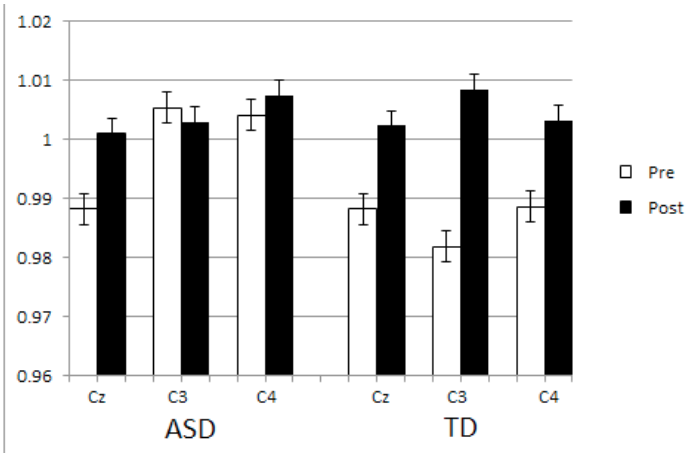
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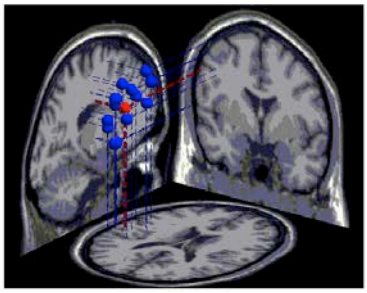
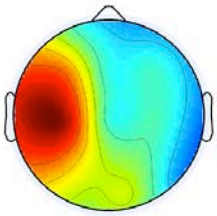
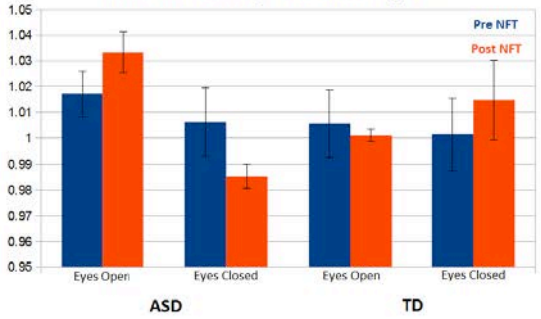


Mu Component In Source Space



Mu Power Changes in Source Space



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Functional connectivity of two key social brain networks in autism spectrum disorder

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Abstract

Converging evidence indicates that brain abnormalities in autism spectrum disorders (ASD) involve atypical network connectivity. Utilizing resting fMRI, this study examined functional connectivity (FC) in two brain networks implicated in social processing – the mentalizing, or theory of mind (ToM), and the mirror neuron system (MNS) – in 25 adolescents with ASD and 25 typically developing (TD) adolescents between the age of 11 and 18 years. Results of whole-brain FC analysis revealed that adolescents with ASD, relative to controls, showed a mixed pattern of both over- and underconnectivity in the ToM network, which was associated with greater social impairment. Further, a secondary analysis comparing a subset of the 15 ASD participants with most severe symptomatology and a tightly matched subset of 15 TD controls revealed exclusive overconnectivity effects, in both ToM and MNS networks, which, likewise, were associated with greater social dysfunction. Finally, greater connectivity in ASD was detected primarily between the regions of the MNS and ToM, suggesting that excessive ToM-MNS cross-talk might be associated with impaired social functioning. Overall, these findings implicate atypically increased connections involving the mentalizing and mirror neuron systems in the social impairments observed in ASD.

Keywords: autism spectrum disorders (ASD); functional connectivity MRI (fcMRI); mentalizing; theory of mind (ToM); mirror neuron system (MNS)

Humans are an inherently social species. Our survival and success depends on our ability to navigate and thrive in complex social situations. This core ability is commonly impaired in autism spectrum disorder (ASD), a neurodevelopmental disorder affecting as many as 1 in 88 children (CDC, 2012). Despite the highly heterogeneous manifestation of core symptoms, impairments in social functioning – such as diminished social responsiveness, difficulty relating to others and recognizing other people's emotions, thoughts and intentions – are a defining feature of ASD (APA, 2000). These core deficits in social functioning have been proposed to be the most universal and specific characteristics of ASD (Sigman et al., 2004), both defining and distinguishing ASD from other developmental disorders (Tager-Flusberg, 2010). Yet, the neural substrates or mechanisms underlying social impairments remain largely undetermined, despite attracting a great deal of research.

Currently, the two most prominent accounts of social dysfunction in ASD are rooted in two – debatably related – theories: theory of mind (ToM) and the mirror neuron system (MNS) account. ToM, also known as the mentalizing system, refers to the ability to infer the contents of other people's minds, including their beliefs and intentions. This ability to attribute mental states, or to *mentalize*, appears to be limited, or at the least delayed in ASD (Baron-Cohen et al., 1985; Happe, 1994; Kaland et al., 2002; Minshew et al., 1997), giving rise to the "mindblindness" theory of autism (Frith, 2001). At the cortical level, mentalizing, or ToM processing, is supported by a frontal-posterior network of brain regions, including the medial prefrontal cortex (mPFC), the bilateral temporal-parietal junction (TPJ), and the posterior cingulate cortex/precuneus (PCC; cf. Amodio & Firth, 2006; Frith & Frith, 2003; Saxe et al., 2009). The other dominant theory, the MNS account, posits that human (and primate) brains possess 'mirror' mechanisms that allow us to understand meaning of the actions and emotions of others by internally replicating them (Gallese et al., 2004). According to this view, we directly experience similar emotions and simulate actions carried out by others, as we observe them doing so. This fundamental 'mirroring' process, inferred from the original discovery in macaques of multimodal neurons firing both during action execution and observation (Rizzolatti et al., 1996), has been proposed as a unifying neural mechanism underlying the understanding of others' actions and emotions and, thus, as a crucial element of human social cognition. The human MNS consists of the anterior intraparietal sulcus (aIPS; also referred to as the rostral inferior parietal lobule, IPL), premotor cortex (PMC; also referred to as the caudal sector of the inferior frontal gyrus, IFG), and posterior superior temporal sulcus (pSTS; Decety & Lamm, 2007; Gallese et al., 2004; Turella et al., 2009). Evidence showing that imitation, a behavioral correlate of the MNS (Iacoboni et al., 1999), is impaired in ASD (as reviewed in Williams et

al., 2004) has given rise to the dominant theory that atypical neural representation of the self-other mapping supported by the MNS may be a key to understanding the nature of social deficits in ASD (Oberman & Ramachandran, 2007; Rizzolatti & Fabbri-Destro, 2010; Williams et al., 2001; although see Dinstein et al., 2010 and Sevlever & Gillis, 2010 for alternative views).

While some authors argue that ToM and the MNS systems are largely independent (e.g., Saxe, 2005), others adhere to a more integrative view, whereby the two brain networks are interdependent, despite recruiting different brain areas (e.g., Ohnishi et al., 2004; Pineda & Hecht, 2009; Uddin et al., 2007). A meta-analysis of more than 200 fMRI task-based activation studies (Van Overwalle & Baetens, 2009) determined that the MNS and ToM are two distinct systems, each involved in processing different aspects of understanding others: while the MNS is activated only in the presence of biological motion (i.e., when moving body parts are observed), ToM is recruited during a more abstract processing of understanding others (i.e., in the absence of biological motion). Except for rare instances, these two systems are seldom concurrently active. While it is understood that judging others in real world likely involves both ToM and MNS systems, the functional distinction between them determined by this meta-analysis was adapted in the present study.

While neuroimaging and electrophysiological evidence suggests that ASD is associated with reduced activation in certain ToM (e.g., Castelli et al., 2002; Spengler et al., 2010) and MNS brain areas (e.g., Dapretto et al., 2006; Hadjikhani et al., 2007; Oberman et al., 2005; Williams et al., 2006), it is also becoming increasingly evident that ASD is characterized by abnormal brain connectivity throughout the brain (Belmonte et al., 2004; Müller, 2007; Schipul et al., 2011; Vissers et al., 2012; Wass, 2011), presumed to stem from altered neurodevelopmental trajectories (Amaral et al., 2008; Courchesne et al., 2007). Widespread abnormalities in interregional connections in ASD have been predominantly demonstrated in functional connectivity magnetic resonance imaging (fcMRI) studies assessing functional relationships between distributed brain regions (for review see Vissers et al., 2012). In fcMRI, functional connectivity is inferred from temporal correlations between fluctuations of the blood oxygen level-dependent (BOLD) signal in spatially distributed networks. Such correlations, reflecting inter-regional coordination, are present even at rest, in the absence of an overt cognitive task (for review see Fox & Raichle, 2007). Importantly, these resting-state connectivity patterns correspond to brain networks recruited during specific cognitive or mental processes (Allen et al., 2011; Beckmann et al., 2005; Smith et al., 2009; Simmons & Martin, 2012) and are therefore considered to represent intrinsically organized functional networks (Fox et al., 2005).

Further, resting-state functional connectivity patterns appear largely consistent with anatomical connectivity (Greicius et al., 2009; Honey et al., 2009) and are robust and highly reliable across individuals (Damoiseaux et al., 2006; Honey et al., 2009; Shehzad et al., 2009; Van Dijk et al., 2010; Zou et al., 2010). One plausible account of the resting correlations is that they reflect a long history of frequent coactivation associated with functional specialization (Fair et al., 2007; Fair et al., 2009).

A growing number of studies have successfully applied rs-fcMRI to examine functional network connectivity in ASD, primarily focusing on the default mode network (DMN), with the most consistent finding indicating reduced FC within the regions of the DMN (Assaf et al., 2010; Kennedy & Courchesne, 2008; Weng et al., 2010), in the face of some reports of overconnectivity or mixed findings (Monk et al., 2009). Lately, the application of rs-fcMRI in ASD has gradually expanded to examining functional networks other than the DMN, such as the salience network incorporating the insula (Ebisch et al., 2011; von dem Hagen et al., 2012) and the striatum (Di Martino et al., 2011). Overall, an important advantage of assessing connectivity in the task-free resting state is the absence of constraints associated with underlying differences in task performance, or task execution strategies, which might present serious confounds in participants with ASD.

The present study investigated whether individuals with ASD show altered functional connectivity (FC) in two brain networks involved in social processing – the mentalizing (ToM) and the MNS systems – and putatively impaired in ASD, by using resting state fcMRI (rs-fcMRI) to examine BOLD fluctuations in regions associated with these networks. Our aims were two-fold: first, to examine functional specialization, as deduced from the functional connectivity of the ToM and MNS brain networks in adolescents with ASD by performing whole-brain connectivity analyses of rs-fMRI data using seed regions identified in a meta-analysis of ToM and MNS activation studies (van Overwalle & Baetens, 2009); and secondly, to relate FC of these networks involved in understanding others to variation on clinical measures of social impairment. It was hypothesized that individuals with ASD would exhibit aberrant connectivity within and between these networks, compared to matched TD controls, and that those with greatest social impairments within the ASD group would show the most atypical connectivity patterns.

Methods

Participants. Thirty adolescents with ASD and 26 typically developing (TD) adolescents, between 11 and 18 years of age, were enrolled in the study. After excluding five participants with ASD due to excessive head

motion (>15% of time points censored; see details below) and one TD adolescent due to hardware malfunction, the final sample included 25 ASD and 25 TD participants matched for age, handedness and non-verbal IQ (see Table 1 for participant characteristics). ASD diagnoses were established using the Autism Diagnostic Interview-Revised (ADI-R; Rutter et al., 2003), the Autism Diagnostic Observation Schedule (ADOS; Lord, 2000), and expert clinical judgment according to DSM-IV criteria (APA, 2000). History of autism-related medical conditions (e.g., epilepsy, Fragile-X, tuberous sclerosis) served as an exclusionary criterion. Inclusion in the TD group required absence of reported personal or family history of autism, and absence of personal history of any other neurological or psychiatric conditions. All participants were native English speakers and had verbal and nonverbal IQ scores greater than 70, as assessed by the Wechsler Abbreviated Scale of Intelligence (WASI; Wechsler, 1999). In addition to the ADI- and ADOS-derived indices of social behavior available only for ASD participants, social functioning was further assessed in all participants using the Social Responsiveness Scale (SRS; Constantino & Gruber, 2005), an informant-based rating scale administered to the participants' parents. Hand preference was assessed with the Edinburgh Handedness Inventory (Oldfield, 1971). Informed assent and consent was obtained from all participants and their caregivers in accordance with the institutional review boards of the University of California, San Diego and San Diego State University.

MRI data acquisition. Imaging data were acquired on a GE 3T MR750 scanner (GE Healthcare, Waukesha, WI) with an 8-channel head coil. High-resolution anatomical images were obtained, for registration purposes, using a standard T1-weighted fast SPGR sequence (TR = 11.08 ms; TE = 4.3 ms; flip angle = 45°; field of view [FOV] = 256 mm; 256 x 256 matrix; 180 slices; 1 mm^3 resolution). Functional T2*-weighted images were acquired with a single-shot gradient-recalled, echo-planar pulse sequence. One 6:10 minute resting-state scan consisting of 185 whole-brain volumes was acquired (TR = 2000 ms; TE = 30 ms; flip angle = 90°; FOV = 220 mm; 64 x 64 matrix; 3.4 mm^2 in-plane resolution; 3.4 mm slice thickness). Throughout the scan, a white fixation cross on a black background was displayed in the center of a screen. Participants were instructed to simply rest in the scanner while keeping their eyes on the cross and staying as still as possible.

fMRI data preprocessing. Images were processed primarily using the Analysis of Functional NeuroImages (AFNI) suite (Cox, 1996; afni.nimh.nih.gov). The first five frames were discarded to remove signal equilibration effects, resulting in 180 total whole brain volumes. The functional data were slice-time corrected for interleaved acquisitions and motion corrected by realigning to the first time point, field-map corrected to remove distortions

resulting from magnetic field inhomogeneity, co-registered to the anatomical image using a single transformation matrix, resampled to 3.0 mm isotropic voxels, standardized to the N27 Talairach template (Talairach & Tournoux, 1988), and spatially smoothed with an isotropic Gaussian filter to an effective full-width at half-maximum (FWHM) of 6 mm. The resulting images were then band-pass filtered at $.008 < f < .08$ Hz to isolate frequencies at which intrinsic network-specific BOLD correlations predominate (Cordes et al., 2001; Fox & Raichle, 2007).

To minimize the confounding effects of head motion on BOLD correlations (Power et al., 2012; van Dijk et al., 2012), several procedures beyond the conventional motion correction were utilized. First, six rigid-body motion parameters (three rotations, three translations) estimated from realignment of functional volumes were modeled as nuisance variables and removed with regression, as detailed below. Additionally, time points with excessive head motion (head displacement >1.5 mm, computed as the root sum of square of displacement between any two time points) and their immediately preceding and following time points were censored from further analyses; blocks of time points with less than 10 usable consecutive images were also excluded. Based on this criterion, the mean percentage of data censored from all 50 participants was <1%. Percentage of data censored did not differ between groups (M_{ASD} = 0.71%; M_{TD} = 0.67%; t (1,48) = 0.06, p = 0.95). Further, to estimate total motion in each participant, the root mean square of displacement (RMSD) across the entire time series was calculated. There was no significant group difference in average total motion as measured by RMSD (M_{ASD} = 0.133; M_{TD} = 0.125; t (1,48) = 0.23, p = 0.82). Finally, the average total motion was not significantly correlated with age (p = .13) or full scale IQ (p = .16).

To remove nuisance BOLD signal attributable to physiological (cardiac and respiratory) artifacts and motion effects, the mean white matter and ventricular signals and six motion parameters were regressed from each participant's 4-D volume data. The white matter and ventricular signals were extracted from the masks derived from Freesurfer's automated segmentation of the anatomical image into tissue compartments, and reduced by one voxel in all directions to prevent partial volume effects with grey matter (Fischl et al., 2002). All nuisance regressors were band-pass filtered at $.008 < f < .08$ Hz.

ToM and MNS regions of interest. Seeds were placed in regions found to be consistently activated by mentalizing or mirror neuron tasks, as determined in meta-analysis of over 200 fMRI studies (van Overwalle & Baetens, 2009). ToM seeds included the mPFC (Talairach coordinates 0 50 20), the bilateral TPJ (±50 –55 25), and the PCC (0 –60 40). Seeds forming the MNS network were the bilateral aIPS (±40 –40 45), pSTS (±50 –55 10), and PMC (±40 5 40). Seeds were created using the Talairach-Tournoux Stereotaxic Atlas (TT-Daemon) in AFNI and

were resampled to the resolution of the fMRI images. Each seed consisted of a 6 mm radius sphere and covered 33 voxels in 3 mm^3 space (see Figure 1, left panel for seed placements and coordinates).

fcMRI analyses. Following fMRI preprocessing and the removal of nuisance variables, the average BOLD time course was extracted from each seed. Next, single-subject FC maps were created by computing Pearson's correlations, *r*, between the mean signal time course for each seed and the time courses of all voxels across the brain (whole-brain voxel-wise correlations). The resulting correlation coefficients were converted to a normal distribution (*z*-values) using Fisher's *r*-to-*z* transformation and were then entered into one- and two-independent-sample(s) *t*-tests to examine within- and between-group FC effects, respectively. All statistical maps were corrected for multiple comparisons to a cluster-size-corrected threshold of $p < 0.05$, using Monte Carlo simulation (Forman, 1995).

Finally, to examine relationships between social impairment and functional connectivity, the mean *z* was calculated for each ROI pair, for every participant, and within- and between-network connectivity indices were computed by averaging mean *z* scores for all within- and between-network ROI pairs, respectively (e.g., the ToM within-network connectivity index was calculated by averaging *z* scores for mPFC-rTPJ, mPFC-lTPJ, mPFC-PCC, PCC-rTPJ, PCC-lTPJ, and rTPJ-lTPJ correlations). These summary indices for within- and between-network connectivity were then correlated with four *a priori* selected social functioning measures, including three diagnostic indices (two ADI-R socio-communicative components: Social and Communication, and the ADOS Communication + Social [CS] total score) and one sociability score available for all (TD and ASD) participants (SRS Total).

Results

Results from the whole-brain functional connectivity analyses performed for each of the 10 seeds are summarized in Figure 1 by way of binarized within-group conjunction maps, separated into the MNS and ToM networks (detailed descriptions of the within-group connectivity results, including peak coordinates, are found in Supplementary Material, Tables S1 and S2). Direct group comparisons (corrected $p < .05$) revealed no significant between-group differences in functional connectivity for any of the MNS seeds, but several significant clusters of differential connectivity for the ToM network, including reduced connectivity of the bilateral TPJ and increased connectivity of the mPFC and PCC in ASD participants (Table 2). Specifically, underconnectivity (TD > ASD) was detected between the bilateral TPJ and the bilateral superior temporal gyri (STG) and PCC/PC, while

overconnectivity (ASD > TD) was detected between the mPFC and PCC, superior parietal lobule (SPL) and middle temporal gyrus (MTG), as well as between the PCC and the middle temporal and inferior frontal gyri (MTG and IFG; Figure 2A and Table 2).

Next, mean *z* scores extracted separately from clusters showing significant over- and underconnectivity for ToM seeds (total of 2 scores for each participant) were entered into correlational analyses with four *a priori* selected social measures (ADI-Social, ADI-Communication, and ADOS Communication + Social [CS] scores available for ASD participants only, and SRS Total available for all participants). The results revealed significant correlations between the extent of ToM over- and underconnectivity and social symptomatology as measured by the SRS Total scores (Table 3). Namely, greater overconnectivity of the PCC and mPFC (found to be overconnected in the ASD group), as well as greater underconnectivity of the bilateral TPJ (found to be underconnected in the ASD group) were both associated with less typical social behavior measured within the entire cohort of participants, combining TD and ASD groups ($r = .67$, $p < .001$ and $r = -.69$, $p < .001$, respectively; see Figure 2A, right panel). In the ASD group alone, connectivity in ToM overconnected clusters was correlated with ADI-Social and ADI-Communication scores, $r = .45$, $p < .05$ and $r = .51$, $p < .01$, respectively; however, neither these nor any other correlations survived Bonferroni correction for multiple comparisons (as detailed below).

While no significant group differences were detected for the MNS network, its functional connectivity strength indexed by mean *z* score, as well as the ToM-MNS between-network connectivity – computed by averaging mean *z* scores for all between-network ROI pairs – were also entered into correlational analysis with the social indices (producing a correlational matrix of 4 connectivity indices x 4 social measures, yielding a Bonferroni adjusted $p < .0.05/16 = 0.003$). As seen in Table 3, despite the absence of significant between-group connectivity differences in the MNS, its average connectivity was positively correlated with ADI-Social scores ($r = .50$, $p = .013$, uncorrected) such that greater MNS connectivity was associated with more severe social symptoms of ASD. Further, the ToM-MNS between-network connectivity was significantly correlated with the severity of social symptoms as measured by ADI-Social ($r = .58$, $p < .003$), indicating that greater ToM-MNS cross-talk was associated with more severe social impairment.

Based on these positive relationships between symptom severity and MNS and ToM-MNS functional connectivity, a post hoc FC analysis was performed in a subset of ASD participants ($n = 15$) with highest level of social symptomatology as defined by ADOS Communication + Social (CS) scores ≥ 10 (see Supplemental Figure

S1 for within-group connectivity maps). Direct group comparison of this ASD subsample and 15 TD participants optimally matched on age, motion, and IQ (see Table S3) corroborated the previous results of increased connectivity (ASD > TD) of the mPFC and PCC seeds with the middle and inferior frontal gyri, but also revealed increased – rather than weaker – connectivity (ASD > TD) of the right TPJ with left middle frontal gyrus (Figure 2B; Table 4). Left TPJ yielded no significant clusters in this direct subsample comparison. However, this analysis yielded a significant between-group difference in the MNS network – which was absent in the direct comparison of total samples – with greater connectivity (ASD > TD) between right aIPS seed and left superior frontal gyrus and PCC (Figure 2B; Table 4). Finally, consistent with analyses for the entire cohort, correlational analyses for these subsamples revealed positive correlations between greater ToM overconnectivity mean *z* scores and SRS Total scores ($r = .66$, $p < .001$), as well as between greater MNS overconnectivity mean *z* scores and SRS Total scores ($r = .74$, $p < .001$; see Figure 2B, right panel).

Discussion

We used resting state fcMRI to investigate the functional connectivity (FC) in two brain networks crucial for social processing (ToM and MNS) in adolescents with ASD, relative to TD controls. In contrast to previous findings of predominantly reduced connectivity in ASD detected at rest in other functional networks (e.g., Assaf et al., 2010; Kennedy & Courchesne, 2008; von dem Hagen t al., 2012; Weng et al., 2010), a mixed pattern of both over- and underconnectivity was observed in the ToM network. Namely, relative to the TD participants, adolescents with ASD showed enhanced connectivity between a seed in mPFC and the superior parietal lobule (SPL), precuneus (PC), and right posterior middle temporal gyrus, as well as between a seed in PCC and the right middle and inferior frontal gyri. On the other hand, the ASD group showed weaker connectivity between the bilateral TPJ seeds and PCC and superior temporal gyrus, including pSTS.

An unexpected finding was the lack of significant between-group differences in the functional connectivity of the MNS network. However, when directly comparing a subset of the ASD group with more severe socio-communicative symptoms and a matched TD subsample, overconnectivity was detected between the raIPS seed region of the MNS and PCC, as well as the raIPS and the left superior frontal gyrus. This secondary analysis involving only the ASD participants with greatest symptom severity also revealed atypically increased connectivity for three ToM seeds (rTPJ, mPFC, and PCC). Specifically, increased connectivity was observed between the right

TPJ and the left middle frontal gyrus, between mPFC and the bilateral superior and middle frontal gyri, and between PCC and the right middle frontal gyrus and left IFG. Remarkably, no underconnectivity effects were observed for this more homogeneous ASD subsample with greater social symptom severity; instead, increased connectivity in the ASD subsample was detected for both MNS and ToM networks. These findings appear inconsistent with the theory of generally reduced long-distance connectivity (Minshew & Williams, 2007) in ASD, or the more specific hypothesis of fronto-parietal underconnectivity (Schipul et al., 2011).

Close examination of the regional specificity of these findings – observed both in the entire sample and in the subset of participants with more severe symptomatology – revealed that atypical connectivity in ASD occurred *between* the regions of the MNS and ToM: for instance, in the analysis of the entire sample, the bilateral TPJ used as ToM seeds showed reduced connectivity with the superior temporal gyrus, including the pSTS region of the MNS, which, in fact, served as one of the seeds in the FC analysis of the MNS. Similarly, clusters found to be overconnected with precuneus – a ToM seed – contained IFG, a canonical MNS region. Likewise, in the analysis of the subset of 15 ASD and 15 TD participants, clusters that emerged as significantly overconnected in both MNS and ToM networks also contained regions from the other network, as detailed in Table 4; for instance, the raIPS seed of the MNS was found to have greater connectivity with the PCC region of the ToM. This pattern of atypical ToM-MNS cross-talk suggests that the two social brain networks putatively impaired in ASD are less distinct, or functionally segregated from one another in adolescents with ASD, at least in the absence of an explicit task. This is in contrast with typical development, during which functional brain networks become simultaneously more integrated (by strengthening of the within-network connections) and segregated (by weakening of the between-network connections; e.g., Dosenbach et al., 2010; Fair et al., 2009; Supekar et al., 2009). Thus, the excess of ToM-MNS connectivity observed in ASD may reflect immature or aberrant developmental processes in two brain networks involved in processing of understanding of others. Moreover, the finding of reduced ToM-MNS functional differentiation is consistent with recently emerging evidence of reduced network segregation in ASD (Shih et al., 2011; Rudie et al., 2012, 2013).

Overconnectivity was most pronounced for a subsample of the 15 ASD participants with highest symptom severity (Figure 2). As one possibility, cross-talk between ToM and MNS, which largely accounted for the overconnectivity effects, might reflect a compensatory mechanism involving strengthening of the atypical connections secondary to social deficits. Specifically, the dynamic nature and complexity of social stimuli and

social interactions may be overtaxing for inefficient neural networks in ASD; as a result, overconnectivity may be a consequence of an over-utilization of aberrant social circuits. The observed links between ToM-MNS cross-network connectivity and socio-communicative symptom severity (on ADI-R Social and Communication scores) may support this interpretation. At the very least, these findings suggest that connectivity of (and between) the ToM and MNS networks plays a role in autistic symptomatology.

The detection of ToM overconnectivity in ASD is particularly noteworthy given the findings indicating reduced activation in the key ToM regions in ASD (e.g., Castelli et al., 2002; Kana et al., 2009; Kennedy & Courchesne, 2008). On the other hand, greater ToM connectivity in ASD might be in line with the evidence that mentalizing brain regions show reduced specialization in autism. Namely, ToM regions have been found to be equally hypoactivated for both mentalizing and physical judgments (Lombardo et al., 2011), and to show activation for tasks that do not pertain to ToM, such as emotional self-assessment (Wang et al., 2007) or irony understanding (Silani et al., 2008), both of which are incidentally impaired in ASD. The ToM network is considered crucial for maneuvering in social contexts, as it supports the understanding of other people's intentions and beliefs. Our finding of ToM overconnectivity in ASD, especially in participants with greater symptom severity, may indicate a state of heightened activity associated with reduced efficiency, consistent with extensive behavioral evidence of ToM impairment in ASD (for review see Frith, 2001; Tager-Flusberg, 2007).

The correlational findings discussed above also supported our second hypotheses regarding links between functional connectivity and social symptom severity in the ASD cohort. Atypical connectivity – reflected in a mixture of under- and overconnectivity for the ToM in the entire sample, but exclusive overconnectivity for both MNS and ToM in a subsample with greater social symptoms – was associated with the SRS Total scores, indicating that those with greater social impairment had more atypical patterns of connectivity. The SRS is a scale specifically designed to quantify impaired social functioning (Constantino, 2002); hence, the robust correlations found between the SRS scores and the abnormalities in the neural circuitry involved in ToM and the MNS indicate that aberrantly increased connections within and between these networks are linked to impaired social functioning. Moreover, increased socio-communicative symptoms in the ASD group, as measured by the ADI-R Social and Communication scales, were linked to the excessive – rather than reduced – connectivity between the two social networks, consistent with the notion that social dysfunction is associated with inadequate segregation between these networks (Shih et al., 2011).

While our findings indicate links between ToM and MNS connectivity and social impairment in ASD, they cannot establish causality. Atypical functional connectivity of these networks could reflect neurobiological abnormalities contributing to the emergence of social impairment. However, alternatively, abnormal social development in children with ASD may result in aberrant connectivity. This latter possibility is supported by evidence that network connectivity, as detected in correlated low-frequency BOLD fluctuations, is affected by learning and experience-driven plasticity (Hasson et al., 2009; Lewis et al., 2009; Stevens et al., 2010). Our findings may also reflect a combination of early causative and secondary, experience-driven effects. Notable in this context was the absence of correlations between connectivity measures and ADOS socio-communicative scores, which represent current abilities, contrasted by stronger correlations between connectivity and ADI-R scores representing the early history of socio-communicative impairment. While caution is required, given the non-experimental nature of these diagnostic scores, the pattern of findings could suggest that at least some of the atypical ToM and MNS connectivity observed here may reflect neural abnormalities possibly contributing to the early emergence of the disorder.

Some limitations of the present study should be noted. Only high-functioning children and adolescents with ASD could be included, and it cannot be determined whether our findings also apply to the lower end of the autistic spectrum. The reasons for this limitation lie in the extreme sensitivity of fcMRI to head motion (Power et al., 2012; Van Dijk et al., 2012). While head motion is clearly also an issue in the study of high-functioning children, we used a number of procedures beyond conventional motion correction to minimize the effects of head movement.

In sum, the current results suggest that atypical connectivity of and between ToM and MNS networks, predominantly reflected in overconnectivity, plays an important role in social processing in children and adolescents with ASD. Moreover, the extent of the atypical connectivity was correlated with greater social impairment, suggesting that abnormal neural connections involving the mentalizing and mirror neuron systems are related to the social impairments observed in ASD.

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Table 1. Participant Characteristics

		ASD (n = 25)		TD (n = 25)		
Gender (M/F)		22/3		20/5		
Handedness (R/L)		23/3		21/4		
		M (SD)	range	*M* (SD)	range	*p* value
Age (years)		14.8 (1.8)	11.8-17.7	14.4 (1.5)	12.1-16.8	0.40
Verbal IQ		111 (15)	83-145	106 (10)	87-126	0.18
Non-verbal IQ		111 (16)	70-140	108 (11)	86-129	0.38
Full-scale IQ		113 (15)	81-141	108 (10)	88-128	0.16
ADOS	Communication	2.9 (1.4)	0-6	n/a		--
	Social Interaction	7.6 (3.2)	1-13	n/a		--
	Repetitive Behavior	2.0 (1.4)	0-5	n/a		--
ADI-R	Social Interaction	16.5 (6.2)	6-25	n/a		--
	Communication	12.6 (6.2)	2-25	n/a		--
	Repetitive Behavior	6.0 (2.3)	3-11	n/a		--
SRS, Total		78.5 (9.8)	58-94	41.5 (5.1)	35-52	<0.001

Note: IQ, intelligence quotient; ADOS, Autism Diagnostic Observation Schedule; ADI-R, Autism Diagnostic Interview-Revised; SRS, Social Responsiveness Scale

Table 2. Regions Exhibiting Group Differences (ASD vs. TD) in Functional Connectivity, separately for MNS and ToM seeds

	Seed	Peak Location	Talairach coordinates x	y	z	Cluster Volume (μl)	T-score
MNS	laIPS	*none*					
	raIPS	*none*					
	lPMC	*none*					
	rPMC	*none*					
	lpSTS	*none*					
	rpSTS	*none*					
ToM	lTPJ	L Superior Temporal Gyrus / pSTS	-50	-28	12	918	4.64
		R PCC	20	-34	26	918	3.75
	rTPJ	L Superior Temporal Gyrus / pSTS	-56	-26	12	2808	4.60
		R/L PCC	2	-44	50	2322	4.46
		R Superior Temporal Gyrus / pSTS	46	-20	14	1485	4.47
	mPFC	L PCC / SPL	-14	-62	50	1755	-4.82
		R Middle Temporal Gyrus	46	-50	20	810	-4.33
	PCC	R Middle Frontal Gyrus, IFG	38	26	38	999	-5.17

Note: laIPS = left anterior intraparietal sulcus; raIPS = right anterior intraparietal sulcus; lPMC = left premotor cortex; rPMC = right premotor cortex; lpSTS = left posterior superior temporal sulcus; rpSTS = right posterior superior temporal sulcus; lTPJ = left temporal-parietal junction; rTPJ = right temporal-parietal junction; mPFC = medial prefrontal cortex; PCC = posterior cingulate cortex/precuneus; SPL = Superior Parietal Lobule; IFG = Inferior Frontal Gyrus; MNS: mirror neuron system; ToM: theory of mind; L: left; R: right.

Table 3. Correlations between connectivity indices and social symptoms measures.

	ADOS-CS[#]	ADI-R, Social[#]	ADI-R, Comm.[#]	SRS, Total[##]
ToM Overconnectivity (PCC, mPFC)	-.29	.45*	.51**	.67***
ToM Underconnectivity (bilateral TPJ)	-.14	.22	.05	-.69***
MNS Connectivity	-.10	.50*	.47*	-.10
ToM-MNS Between-Network Connectivity	-.07	.58**	.57**	.11

Note: ***p < .003 (Bonferroni corrected p <. 05); **p < .01 (uncorrected); *p < .05 (uncorrected);
[#] correlation coefficients are calculated for n = 25; [##] correlation coefficients are calculated for N = 50.

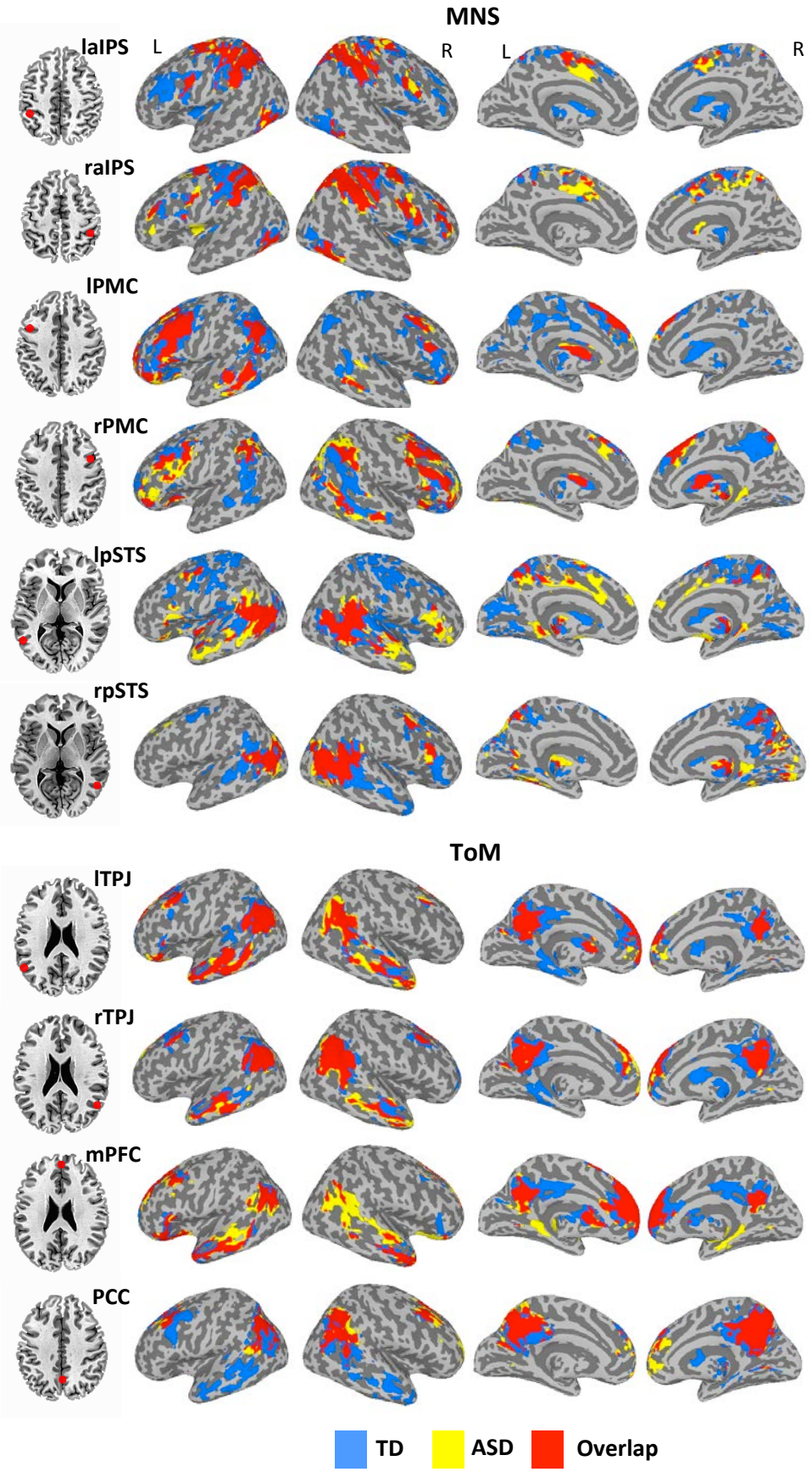
Table 4. Regions Exhibiting Group Differences in Functional Connectivity, in a subsample of 15 ASD participants with ADOS-CS ≥ 10 and 15 TD controls, for MNS and ToM seeds.

	Seed	Peak Location	Talairach coordinates x	y	z	Cluster Volume (μl)	T-score
	laIPS	*none*					
	raIPS	L Superior Frontal Gyrus	-16	26	48	1296	-4.41
		R/L PCC	2	-38	36	837	-3.86
MNS	lPMC	*none*					
	rPMC	*none*					
	lpSTS	*none*					
	rpSTS	*none*					
	lTPJ	*none*					
	rTPJ	L Middle Frontal Gyrus	-16	46	30	756	-4.30
ToM	mPFC	L Middle / Superior Frontal Gyrus	-26	-10	44	2808	-4.12
		R Superior / Middle Frontal Gyrus	28	-8	56	1944	-4.70
	PCC	L IFG, p.Tri/p.Op	-52	14	18	1242	-4.44
		R Middle Frontal Gyrus	34	22	38	1269	-4.66
		L IFG, p.Tri/p.Orb	-46	40	2	810	-4.51

Note: laIPS = left anterior intraparietal sulcus; raIPS = right anterior intraparietal sulcus; lPMC = left premotor cortex; rPMC = right premotor cortex; lpSTS = left posterior superior temporal sulcus; rpSTS = right posterior superior temporal sulcus; lTPJ = left temporal-parietal junction; rTPJ = right temporal-parietal junction; mPFC = medial prefrontal cortex; PCC = posterior cingulate cortex/precuneus; IFG = Inferior Frontal Gyrus; MNS: mirror neuron system; ToM: theory of mind; L: left; R: right.

Figure 1. Within-group functional connectivity maps for MNS (top panel) and ToM (bottom panel) seeds. Results of the within-group (ASD, TD; $p < .05$ corrected) analyses obtained for each MNS and ToM seeds (top and bottom panels, respectively) are presented in a conjunction view. Seed ROIs are presented on the axial slices on the left. Inflated maps were generated using Surface mapping with AFNI (SUMA; http://afni.nimh.nih.gov/afni/suma). laIPS = left anterior intraparietal sulcus; raIPS = right anterior intraparietal sulcus; lPMC = left premotor cortex; rPMC = right premotor cortex; lpSTS = left posterior superior temporal sulcus; rpSTS = right posterior superior temporal sulcus; lTPJ = left temporal-parietal junction; rTPJ = right temporal-parietal junction; mPFC = medial prefrontal cortex; PCC = posterior cingulate cortex/precuneus; L: left; R: right.

Figure 2. Regions exhibiting group differences (ASD vs. TD) in functional connectivity.
(A) Clusters of significantly different functional connectivity ($p < .05$ corrected) in ASD participants relatively to the TD participants are illustrated for the ToM seeds. Scatterplots on the right show the relationship between the ToM overconnectivity (average *z* scores for mPFC and PCC seeds) and social impairment measured by the SRS Total [$r(50) = .67$, $p < .001$], and between the ToM underconnectivity (average *z* scores for bilateral TPJ seeds) and social impairment measured by the SRS Total [$r(50) = –.69$, $p < .001$].
(B) Clusters of significantly different FC ($p < .05$ corrected) in the subset of 15 ASD participants with ADOS-CS ≥ 10 and 15 matched TD participants. All depicted ToM and MNS seeds yielded overconnected clusters (ASD>TD). Scatterplots on the right show the relationship between the ToM overconnectivity (average *z* scores for mPFC, PCC and rTPJ seeds) and social impairment measured by the SRS Total [$r(30) = .66$, $p < .001$], and between the MNS overconnectivity (mean *z* scores for raIPS) and social impairment measured by the SRS Total [$r(30) = .74$, $p < .001$]. Increasing SRS Total values indicate greater social impairment.


MNS
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TD
ASD
Overlap

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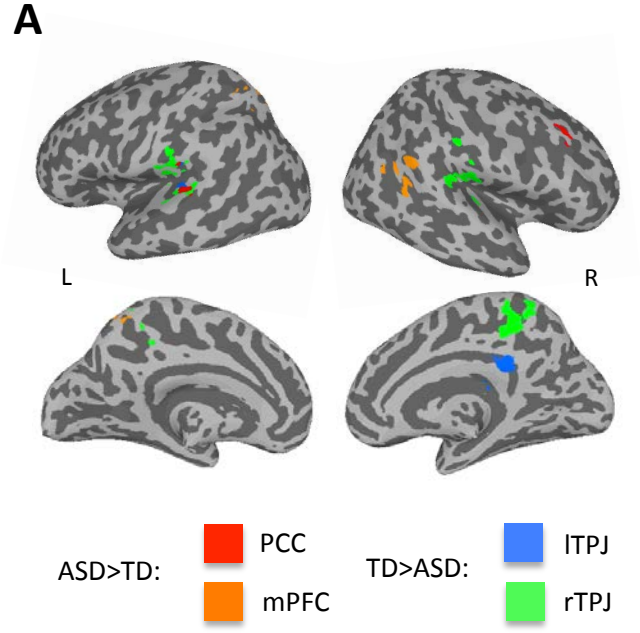
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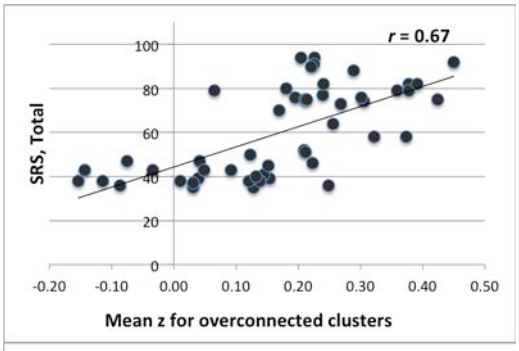
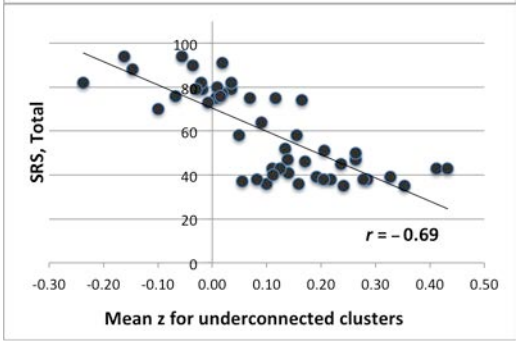
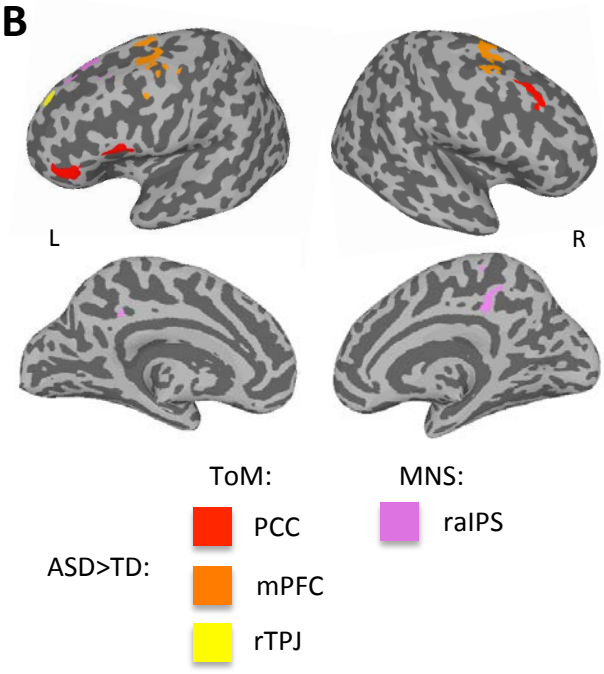
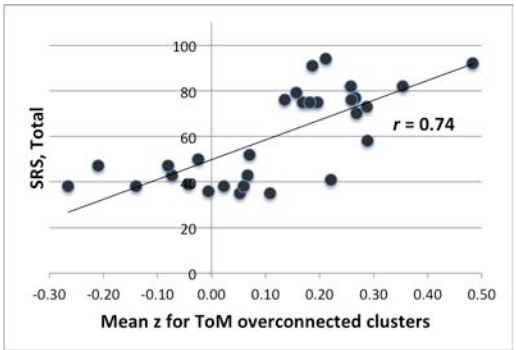

r = 0.67
SRS, Total
Mean z for overconnected clusters


r = −0.69
SRS, Total
Mean z for underconnected clusters

B


L
R
ToM:
PCC
mPFC
rTPJ
ASD>TD:
MNS:
raIPS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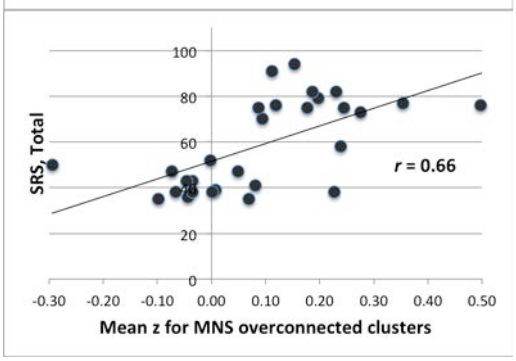
SRS, Total
Mean z for ToM overconnected clusters


r = 0.66
SRS, Total
Mean z for MNS overconnected clusters

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Self-regulation of brain oscillations as a treatment for aberrant brain connections in children with autism

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ABSTRACT

Autism is a highly varied developmental disorder typically characterized by deficits in reciprocal social interaction, difficulties with verbal and nonverbal communication, and restricted interests and repetitive behaviors. Although a wide range of behavioral, pharmacological, and alternative medicine strategies have been reported to ameliorate specific symptoms for some individuals, there is at present no cure for the condition. Nonetheless, among the many incompatible observations about aspects of the development, anatomy, and functionality of the autistic brain, it is widely agreed that it is characterized by widespread aberrant connectivity. Such disordered connectivity, be it increased, decreased, or otherwise compromised, may complicate healthy synchronization and communication among and within different neural circuits, thereby producing abnormal processing of sensory inputs necessary for normal social life. It is widely accepted that the innate properties of brain electrical activity produce pacemaker elements and linked networks that oscillate synchronously or asynchronously, likely reflecting a type of functional connectivity. Using phase coherence in multiple frequency EEG bands as a measure of functional connectivity, studies have shown evidence for both global hypoconnectivity and local hyperconnectivity in individuals with ASD. However, the nature of the brain's experience-dependent structural plasticity suggests that these abnormal patterns may be reversed with the proper type of treatment. Indeed, neurofeedback (NF) training, an intervention based on operant conditioning that results in self-regulation of brain electrical oscillations, has shown promise in addressing marked abnormalities in functional and structural connectivity. It is hypothesized that neurofeedback produces positive behavioral changes in ASD children by normalizing the aberrant connections within and between neural circuits. NF exploits the brain's plasticity to normalize aberrant connectivity patterns apparent in the autistic brain. By grounding this training in known anatomical (e.g., mirror neuron system) and functional markers (e.g., mu rhythms) of autism, NF training holds promise to support current treatments for this complex disorder. The proposed hypothesis specifically states that neurofeedback-induced alpha mu (8–12 Hz) rhythm suppression or desynchronization, a marker of cortical activation, should induce neuroplastic changes and lead to normalization in relevant mirroring networks that have been associated with higher-order social cognition.

Introduction

Autism is a highly varied developmental disorder typically characterized by deficits in reciprocal social interaction, difficulties with verbal and nonverbal communication, and restricted interests and repetitive behaviors. In the current Diagnostic and Statistical Manual of Mental Disorders. 4th ed. (DSM-IV) [1], autism is considered the prototype for the category called pervasive developmental disorders (PDD). Of the pervasive developmental disorders, autistic disorder (AD), Asperger's disorder, and pervasive developmental disorder not otherwise specified (PDD-NOS) are informally referred to as the autism spectrum disorders (ASD). ASD was once considered to be of psychogenic origin but is now widely recognized to be a developmental disorder involving genetic and environmental factors and multiple functional brain networks. Among the many incompatible observations about aspects of the development, anatomy, and functionality of the autistic brain, it is widely agreed that autism is a disorder of connectivity [2,3].

Epidemiological studies show that ASD prevalence rates have been increasing in recent years, with current CDC reports indicating an average rate of about 1% (1/110), with increases of 8–17% per year [4]. While only 68% of the increase can be attributed to increased awareness and updated diagnostic criteria, the remaining 32% of cases represent a real increase in prevalence [5]. Although a wide range of behavioral, pharmacological, and alternative

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medicine strategies have been reported to ameliorate specific symptoms for some individuals (for recent reviews see [6–8]), there is at present no cure for the condition. With no clear biological marker or risk factor associated with the onset of ASD, the inherent heterogeneity of endophenotypical presentation makes clinical management challenging.

In clinical studies, the most effective type of therapy for ASD is behavioral intervention, with an efficacy rate of approximately 48% [9–11]. Unfortunately, like most clinically validated therapeutic approaches for ASD, behavioral therapy is time consuming and costly for such a low potential benefit. Thus, alternative interventions would be beneficial and warrant serious consideration. While the precise mechanisms of neurofeedback (NF) are not yet well understood, the evidence suggests it can capitalize on the implicit plasticity of the brain to induce neural, functional, and ultimately behavioral changes. Furthermore, with the use of quantitative electroencephalography (qEEG) and specific NF protocols (e.g., amplitude and coherence training) for individual subjects, NF can be targeted to fit the heterogeneity of autistic symptoms. Therefore, the present review uses promising observations from a variety of sources to support the hypothesis that NF training is a viable treatment option for autism.

Aberrant connectivity in the autistic brain

The numerous and diverse observations of structural abnormalities in grey and white matter in the autistic brain (see Table 1) have led many researchers to question the specific nature of this apparent aberrant connectivity. The development of functional connectivity magnetic resonance imaging (fcMRI) has largely supported initial observations about neural connectivity derived from anatomical work. Initially studied by Biswal et al. [12], fcMRI measures synchronized fluctuations in BOLD signal activity that, by inference, correlate with the connectivity of networks in the brain [13,14]. Functional connectivity is based on the idea that cognitive and social capabilities emerge from the collaborative activity of large-scale cortical networks, operationally defined by the synchronicity of their hemodynamic activity.

First described by Just et al. [15], the underconnectivity hypothesis of ASD posits that "autism is a cognitive and neurobiological disorder marked and caused by under functioning integrative circuitry that results in a deficit of integration of information at the neural and cognitive levels." Decreases in connectivity in ASD are consistent across studies using various cognitive, emotional, and social tasks [16–18]. While many fcMRI studies have used tasks to demonstrate differences in cortical networks, other studies have used an analysis of the "resting state." This method examines spontaneous fluctuations in hemodynamic activity that appear even in the absence of task performance [19]. Networks that coactivate during task performance often show high within-network correlations of these spontaneous fluctuations even during rest. Many studies show that these correlations are also reflected in structural connectivity measures [20]. These findings support the use of "resting state" connectivity as a proxy for task-related functional connectivity, and in some cases structural connectivity. One consistent finding of these resting state fcMRI studies is a correlated network of regions thought to be involved in introspection, daydreaming, or self-referential thought, commonly known as the "default mode network" [21]. Activation in this network tends to be negatively correlated with goal-directed networks [22]. Across studies, individuals with ASD demonstrate decreased resting state connectivity in the default mode network compared to typically developing controls [23], as well as a reduced "switching" from this network to task-related networks during task performance [24]. Still, a number of studies have amended the original hypothesis, suggesting that while there may be reduced local connectivity, there may actually be increased long-range connectivity [25]. The discrepancies in many fcMRI findings and methodologies have warranted several skeptical meta-analyses [3].

Nonetheless, the recent surge of papers on the topic of connectivity in ASD make it clear that there is *atypical* or *aberrant* connectivity, though it is too early to specify its exact nature. In a recent host of both resting state and task-related fcMRI studies, a general theory of a disordered connectivity has emerged [16,17,23,26–32]. As Müller et al. [3] point out, "Among the few neuroscientific findings that appear solid are those of abnormal white matter growth

Table 1
The neuroetiology of autism spectrum disorder: anatomical markers.

Main finding	Method	Representative publications
Increased head circumference; higher rates of macrocephaly	Anatomical measurements	[143]
Increases in cerebral volume	Magnetic resonance imaging (MRI)	[144–146]
Increases in frontal and temporal gray matter volume	MRI	[147]
Increased neuron counts and brain weight in prefrontal cortex	Post-mortem anatomical analysis	[148]
Gray matter increases in regions related to social cognition, communication, and repetitive behaviors, as well as auditory and visual perception	MRI	[149]
Decreases in parietal lobe volume	MRI	[150]
Lack of asymmetry in planum temporale volume	MRI	[151]
Increased cortical thickness in temporal and parietal lobes		[152]
Decreases in gray matter density in ventromedial aspects of the temporal cortex	MRI	[147]
Cortical thinning in regions related to the mirror neuron system, emotional recognition, and social cognition	MRI	[82]
Increases in local density and computation in cortical minicolumns	Post-mortem anatomical analysis	[153]
Increased white matter growth, especially in the prefrontal cortex and cerebellum	MRI	[144,145]
Increases in the cerebral white matter specifically in the parietal, occipital, and frontal lobes	Transverse relaxation time imaging	[154]
Decreases in corpus callosum volume	MRI, Diffusion tensor imaging (DTI)	[155,156]
Reduced fractional anisotropy in a variety of white matter regions, especially corpus callosum, frontal, and temporal regions	DTI	[34–36,157–159]
Mean diffusion increases in various regions including corpus callosum, arcuate fasciculus, and temporal areas	DTI	[33,34,36]
Increased connectivity volume between the superior temporal sulcus and amygdala	fMRI, DTI	[160]

trajectories and impaired connectivity." To bring some level of reconciliation among these various studies, several investigators have proposed a *local overconnectivity-long range underconnectivity hypothesis* [32] that is supported by noisy local processing in minicolumns [32] and reduced integrity in extensive white matter tracts [33–36].

The neurofeedback hypothesis

Aberrant connectivity in the ASD brain, be it increased, decreased, or otherwise compromised, may complicate healthy synchronization and communication among and within different neural circuits, thereby producing abnormal processing of sensory inputs necessary for normal social life. These anomalies in neural connections could be responsible for the abnormal social behaviors in children with autism. It is hypothesized that neurofeedback, an intervention based on operant conditioning that results in self-regulation of the electroencephalogram produces positive behavioral changes in ASD children and does this by normalizing the aberrant connections within and between neural circuits. Thus, in order to fully address the behavioral symptoms of ASD, it is crucial to understand the gap between connectivity and cognition. Informed by knowledge of the neural underpinnings associated with the social dysfunctions present in ASD, methods such as EEG have made it possible to concurrently measure connectivity between brain regions and corresponding behaviors and to remediate the problem.

The use of EEG biomarkers

It is widely accepted that the innate properties of brain electrical activity produce pacemaker elements and linked networks that oscillate synchronously or asynchronously, likely reflecting a type of functional connectivity [37]. Although the periodicity of such oscillations varies in distinct frequency bands as a function of neural architecture [38], consensus exists that at least three types of oscillating relationships recorded as scalp EEG arise from cortex [39–41]. First, in the columnar architecture of cortex, synchronous activities are created locally between neighboring columns and these "local" oscillations produce high frequency components above 30 Hz, often labeled as gamma rhythms. Synchronization in the gamma band has been proposed as a type of neural binding mechanism that subserves perceptual and cognitive functions [42,43]. Oscillations can also occur between cortical columns separated by a short distance (e.g., several centimeters). These intermediate or "regional" oscillations appear to produce intermediate frequency components such as high alpha/mu (10–12 Hz) and beta components. Finally, oscillations develop between cortical regions that are much further apart, such as frontal and parietal or occipital and frontal cortices. These "global" oscillations are more closely related to slower frequency band components, such as delta (1–4 Hz), theta (4–8 Hz), and low alpha/mu (8–10 Hz). Various types of oscillations can occur spontaneously as a function of non-contingent firing in cellular networks, as part of thalamocortical re-entrant interactions and pacemaker cells that make up thalamic nuclei, or as activity time-locked to extrinsic stimuli during the processing of a task.

Resting state fcMRI findings have been replicated by a limited number of qEEG studies that have also observed decreased resting state connectivity. Similar to fcMRI, qEEG measures the synchronicity of brain networks, but with less spatial precision and higher temporal resolution. Using phase coherence in multiple frequency bands as a measure of functional connectivity, studies have shown evidence for both global hypoconnectivity and local hyperconnectivity in individuals with ASD [44–47]. Several of these studies have noted increased coherence in gamma frequency bands over the parietal [44] and temporal lobes [48], suggesting increased local connectivity. Likewise, Murias et al. [46] found locally elevated coherence in the theta (3–6 Hz) frequency range in ASD subjects, particularly over left frontal and temporal regions. Meanwhile, there was lower coherence in the ASD subjects in the lower alpha range (8–10 Hz) within frontal regions [46]. In a qEEG study with 20 autistic children, Coben et al. [47] found patterns of hypocoherence in ASD, including decreased intrahemispheric delta and theta coherences across short to medium and long inter-electrode distances. Children with ASD had lower interhemispheric delta and theta coherences across the frontal region, and delta, theta and alpha hypocoherence was also evident over temporal regions. In posterior regions, low delta, theta and beta coherence were observed in children with ASD [47]. Thus, while these findings are diverse and multifaceted, there is an emergent framework of local hyperconnectivity and global hypoconnectivity in the autistic brain.

Although the characterization and specific nature of neural connectivity in ASD is incomplete, awareness of the brain's experience-dependent structural plasticity [49,50] suggests that these abnormal patterns may be reversed with the proper type of treatment [51,52]. Plasticity in this case refers to not only the changing of synaptic strengths but to processes that contribute to the homeostasis of network activity. Atypical fcMRI and qEEG results may be the consequence of early aberrations of white matter development and "disturbances in experience-driven network formation through regressive and constructive processes, such as synaptic pruning and stabilization" [3], and may therefore be amenable to additional induction of plasticity.

Self-regulation of EEG oscillations

Of specific interest to neurotherapeutic interventions such as NF is whether brain oscillations are causally implicated in brain function, or whether they are simply epiphenomenological or by-products of other, underlying mechanisms? Animal intracranial recordings and human electrophysiology have shown that neural oscillatory mechanisms are directly related to and play a critical role in a number of cognitive functions including learning, memory, attention, feature binding and sensory selection and gating [38,53,54]. However, less direct evidence exists on the effects, whether short- or long-term, of the modulation or entrainment of these oscillations and their relationship to brain plasticity [55].

As noted previously, brain oscillations are instantiated across different spatial scales [38] from single pacemaker neurons [56], to neuronal circuits [57], to re-entrant thalamo-cortical and large scale cortico-cortical networks [58]. It is assumed that one of the computational processes these oscillations enable is the dynamic routing and gating of information through the synchronization of various elements [59–61]. Indeed, multi-frequency synchronies are thought to be critical for linking spatially distributed neuronal assemblies into functionally integrated and specialized networks, and shown to play a role in sensory registration [62], perceptual integration [63], and selective attention [64]. From a computational perspective, the rhythmic stimulation of the oscillating neural population can be modeled as a periodic force acting in a certain direction on the phase vector [65,66], while from a system level perspective, EEG responses to sensory stimuli can partially be explained through transient, stimulus-induced adjustment of the phase of ongoing oscillations via phase-resetting [67–69].

The possibility of volitional modulation or entrainment of these oscillations raises an interesting set of questions. Is it possible to promote/enhance or inhibit/suppress oscillations in distinct, neuronal elements/networks *in vivo* via indirect "internal" signaling similar to directly stimulating them (e.g., through transcranial stimulation protocols)? In other words, can we modulate these oscillations volitionally through some periodic internal input or

drive? Provided that these oscillations play a causal role for a specific cognitive function, it is at least theoretically plausible that their modulation/entrainment can have a functional impact. A brain computer interface, which allows real-time information of brain activity to be fed back to a user by means of a computer in a closed 'neurofeedback' loop, enables control and natural operation of brain oscillations across cortical networks *in vivo* and in real time [70–72]. Although the specific aims of NF approaches differ, most use a simple visual stimulus or game to train the individual to increase/decrease a certain bandwidth of EEG signal. With training, most individuals can develop a remarkable level of control over his/her brain oscillations. During NF, subjects are exposed to the same visual/auditory feedback or reward stimuli, and hence the entrained EEG differences most likely represent the modulation of some internal or 'background' brain state(s) associated with the event rather than to external factors.

The role of the mirror neuron system

The discovery of mirror neurons in monkeys and a Mirror Neuron System (MNS) in the human brain has provided a neurobiological substrate for understanding many key concepts in human social cognition directly relevant to the behavioral and cognitive deficits observed in ASD [73], including the ability to comprehend actions, glean intentions, and learn through imitation. First described by Rizzolatti and co-workers [74] in the macaque monkey, mirror neurons are thought to be involved in both self-initiated action and the representation of action performed by others. Neurons in the pars opercularis of the inferior frontal gyrus (IFG) show increased firing while executing and observing the same action, representing a potential mechanism for mapping seeing into doing [75,76]. It is well reported that individuals with ASD have marked impairment in social skills, from joint attention to understanding the intentions of others, often termed "mind-blindness" [77,78]. As has been noted in a number of recent reviews, deficits in MNS activity may explain the poor socialization skills prevalent in the disorder.

Although some studies have raised questions about the role of mirror neurons in human social behavior [79,80], an increasing amount of work suggests that a dysfunction in the MNS does contribute to social deficits [81–86]. Specifically, deficits likely arise from an inability to "form and coordinate social representations of self and others via amodal or cross-modal representation processes" [87] – the type of function ascribed to mirror neurons. A particularly striking fMRI study by Dapretto et al. [84] demonstrated decreased activation in the inferior frontal gyrus (pars opercularis) in autistic individuals, and found that activity in this region was inversely related to symptom severity in the social domain. Similarly, EEG studies (described in later sections) have shown that putative electro-biomarkers of MNS activity show abnormalities in ASD compared to typically-developing children [83,86,88,89]. Despite the excitement generated by these observations, few if any investigations have focused on operationalizing insights into MNS function towards practical solutions to the early diagnosis and possible repair of MNS deficits in clinical disorders.

The MNS and mu rhythms

While functional hemodynamic studies have delineated areas in the human brain that might act as analogs to the monkey MNS, direct recording of neural activity by electromagnetic methods have unveiled neural activation patterns correlated with mirroring. Particularly relevant are scalp-recorded EEG patterns of activity in the alpha (8–13 Hz) and beta (15–25 Hz) range that are most evident over the central region of the scalp overlying the sensorimotor cortices and modulated by motor activity [90]. Traditionally these patterns of oscillations have been labeled "mu rhythms" (reviewed by [76]. The major characteristic of the mu rhythms is that they reach maximal power in the absence of overt movements, when the participant is at rest. In fact, mu rhythms are desynchronized and their power reduced when a hand or a foot movement is prepared, and disappears when the movement is actually performed. Initially, these rhythms were considered to be the default rest state of the brain ("idling rhythms," [76,91], present as part of the normal waking state. However, newer data showing different patterns of event-related de-synchronization (ERD) and power suppression have linked these phenomena with cognitive functions such as memory [92–94], selective attention [95,96] as well as affect [97–99]. Particularly relevant to this chapter is evidence for mu suppression not only when participants perform movements but also when they observe such movements [100–102]. During the self-initiation, observation, or even imagination of action in typically developing individuals, the MNS network is active and power in the mu rhythm is suppressed [90,101,103,104].

Indeed, the phenomenology of the mu rhythm resembles the phenomenology of mirror neuron activity. Both are sensitive to movement, as well as to motor and cognitive imagery (i.e., observed meaningful actions). Their overlapping neural sources in sensorimotor frontoparietal networks supports the argument that they are related and involved in linking perception to action, which may be a critical component in the development of social cognition. Mu rhythms appear to reflect the translation of "seeing" and "hearing" into "doing" [76]. This function requires the entrainment of multiple domain-specific generators. These domains exhibit synchronized and desynchronized activity in a locally independent manner but become entrained when they are coherently and globally engaged in translating perception into action [76]. These patterns suggest a link between MNS and mu rhythms and raise the possibility that brain mechanisms manifested by EEG mu rhythms reflect social interaction, including imitation behavior and theory of mind [105]. If so, it stands to reason that the modulation of mu rhythms might be dysfunctional in ASD individuals whose performance in these domains is impaired.

The integration of fMRI and EEG techniques during tasks that activate the MNS have demonstrated that mu rhythm suppression occurs in typical MNS regions, namely the inferior parietal lobe, dorsal premotor cortex, and primary somatosensory cortex [105]. In autistic individuals this mu rhythm suppression is not observed, supporting the role of an altered MNS in ASD [86,106]. Oberman et al. [86] compared mu suppression in a group of 10 individuals with high functioning ASD ranging in age between 6 and 47 years with age-matched typically developing (TD) controls in four different conditions: (a) performing a simple hand movement, (b) observing a video showing the same hand movement performed by the experimenter, (c) observing two balls bouncing, and (d) observing visual white noise (as baseline). As expected, there was no mu suppression for observing non-biological movement in either group; both groups exhibited significant mu suppression while performing the hand movement, but only in the TD group was mu rhythm significantly suppressed in the observe-only condition. These results provide evidence for a defective MNS associated with ASD and have recently been replicated by others [83].

Conclusions

The observations linking brain oscillations to function have important implications for therapies of brain disorders associated with abnormal cortical rhythms, particularly mu rhythms, and support the use of EEG-based NF as a noninvasive tool for establishing a causal link between rhythmic cortical activities and their functions [107]. The proposed hypothesis is that neurofeedback-induced alpha mu (8–12 Hz) rhythm suppression or

desynchronization, a marker of cortical activation [108], should induce neuroplastic changes in relevant networks. In contrast, beta mu (12–15 Hz) synchronization, which has been associated with cortical deactivation [109] and motor inhibition [110], might produce an opposite pattern. With knowledge of the brain's inherent plasticity and with mu suppression as a potential electrophysiological marker of MNS activity, we can use EEG to train the brain to develop volitional control over its waveforms, and by extension, its functional connectivity.

Evaluation of the hypothesis

The treatment of epilepsy using NF training is arguably the best-established clinical application of EEG operant conditioning [110]. Sterman initially described an EEG oscillation with a frequency of 12–20 Hz, similar to EEG sleep spindles, which has been referred to as the "sensorimotor rhythm" or SMR [111]. During the testing of a highly epileptogenic compound, Sterman and co-workers found elevated seizure thresholds in cats that had previously taken part in SMR conditioning, suggesting that the SMR training had somehow predisposed the cats against experiencing seizures. These findings have been successfully extrapolated to humans where it has been documented that seizure incidence is lowered significantly through SMR training [112]. SMR rhythms have been shown to originate in the ventrobasal nuclei (nVB) of the cat thalamus [113], an area involved in the channeling of afferent somatosensory information to cortex. During conditioning, the firing patterns of nVB cells shift from fast and non-rhythmic discharges to systematic, rhythmic bursts that are associated with suppression of somatosensory information flow [113,114]. This reduction causes the nVB cells to hyperpolarize. However, instead of sustaining a stable level of inhibition, the cells begin to gradually depolarize as a function of a slow calcium influx. This eventually causes the nVB neurons to discharge a burst of spikes that is then relayed to sensorimotor cortex and thalamic reticular nucleus (nRT) neurons. Stimulation of the nRT leads to inhibition of VB relay cells, returning them to a hyperpolarized state and initiating a new cycle of slow depolarization producing rhythmic thalamocortical volleys and consequent cortical EEG oscillations [115].

Consistent with the work by Sterman and co-workers, Ros et al. [107] have shown that self-regulation of EEG rhythms in quietly sitting, naive humans significantly affects the subsequent corticomotor response to transcranial magnetic stimulation (TMS), producing durable and correlated changes in neurotransmission. More specifically, the intrinsic suppression of alpha cortical rhythms produced robust increases in corticospinal excitability and decreases in intracortical inhibition of up to 150%, lasting more than 20 min. Likewise, Hinterberger et al., [116] showed that brain regulation of the slow cortical potential (SCP) to activate an external device led to activation of specific brain areas. That is, a successful positive SCP shift compared with a negative shift was closely related to an increase in the BOLD response in the basal ganglia. Successful negativity was related to an increased BOLD response in the thalamus compared with successful positivity. These results indicate learned regulation of a cortico-striatal-thalamic loop modulating local excitation thresholds of cortical assemblies.

Finally, Beauregard and Lévesque [117] scanned 15 unmedicated ADHD children randomly assigned to an experimental group that received NF, and five other ADHD children assigned to the control group who did not receive NF. Subjects from both groups were scanned 1 week before the beginning of training and 1 week after, while they performed a Counting Stroop task. Prior to training, the Counting Stroop task was associated with significant focus of activation in the left superior parietal lobule for both groups but no activation in the anterior cingulate cortex (ACC). Following training, the Counting Stroop task was still associated with increased activation of the left superior parietal lobule for both groups, but for the experimental group only there was a significant activation of the right ACC. The results suggest that NF training has the capacity to normalize the functioning of the ACC in ADHD children. These precedents for NF treatment suggest that it may also be effective in modulating EEG signals associated with deficits in ASD, particularly in the realm of social cognition.

Neurofeedback treatment for ASD

NF as a technique for modifying behavior has been used primarily in clinical settings, and support for its efficacy is based largely on case studies with only a few randomized, controlled, and blinded studies. Nonetheless, a substantive amount of work supports the rationale for NF use in the context of treatment. As previously discussed, there is already evidence supporting the efficacy of this approach for a variety of neuropsychological conditions, including ADHD [118–120], epilepsy [121–125], traumatic brain injury [126,127], anxiety [128], and substance abuse [129].

In terms of ASD, it is well recognized that more than 50% of individuals with ASD demonstrate significant electrophysiological abnormalities on EEG [130–132]. Upwards of 30% develop clinical seizures by adolescence, and even when clinical seizures have not been identified, more than 50% show paroxysmal sharp discharges on EEG, especially during sleep. Additional daytime EEG abnormalities include altered spectral profiles, abnormal patterns of coherence, and reduced mu rhythm activity. These observations have led many clinical practitioners to use EEG-based interventions as a therapeutic strategy.

Cowan and Markham [133] conducted one of the earliest case studies of neurofeedback and autism. QEEG analysis on an eight year-old high functioning female showed abnormally high alpha (8–10 Hz) and theta (4–8 Hz) activity in the posterior regions of the brain. Following more than 20 weeks of NF training, the child showed improvements in sustained attention as assessed by the Test of Variables of Attention (TOVA), decreased autistic behaviors, such as inappropriate giggling, and spinning, and improved socialization based on parental and teacher assessments. Sichel [134] also reported positive changes in all DSM-IV-R diagnostic criteria for autism in a single case study. A few years later, two scientifically controlled studies reported significant reductions in autistic symptoms following NF training. Jarusiewicz [135] reported an average of 26% improvement (sociability (33%), speech/language/communication (29%), health (26%), and sensory/cognitive awareness (17%)) in the ATEC in 12 children diagnosed with autism compared to 3% improvement in a control group. Coben and Hudspeth (cited in [136]) studied 14 ASD children with significantly high levels of mu rhythm activity and a lack of mu suppression during observational activity. Participants were assigned to an interhemispheric bipolar training or a coherence training group designed to increase connectivity between central and peripheral frontal regions via assessment guided NF. Both groups improved significantly on neurobehavioral and neuropsychological measures, but only in the coherence training treatment group was mu activity significantly reduced. Increased coherence was associated with diminished mu and improved levels of social functioning [137].

In a series of two experiments, Pineda et al. [138] examined whether neurofeedback could lessen abnormal mu rhythms and behavioral outcomes in 27 children with high functioning autism. In the first study, eight ASD males were randomly assigned to an experimental or placebo group. NF training included 30 sessions of 30 min each with rewards for mu-like activity (8–13 Hz) and inhibits for EMG (30–60 Hz). The ATEC showed changes (9–13%) in two of the four experimental participants. In the second study, 19 children with verified high functioning ASD were randomly

assigned to an experimental or placebo group. NF training was similar to study one except the reward band was now 10–13 Hz (or high mu band). Parent ratings showed a significant reduction in symptoms as measured by the ATEC Total score, although there was an increase in ratings of Sensory/Cognitive Awareness in excess of 40% that did not occur in the placebo control group, suggesting that participants improved in some areas and regressed in others.

Coben and Padolsky [136] used assessment guided NF on 37 patients over the course of 20 sessions to reduce hyperconnectivity in posterior-frontal to anterior-temporal regions. Following NF, parents reported symptom improvement in 89% of the experimental group, with very little change in the control group. Improvement also occurred in the areas of attention, visual perceptual functioning, language, and executive functioning, with a 40% reduction in core ASD symptoms as assessed by the ATEC total scores. There was also decreased hypercoherence in 76% of the experimental group as measured by a post-training qEEG. The results suggest that decreased hyperconnectivity could have produced the positive changes in treatment outcomes.

In more recent studies, Kouijzer et al. [139] reported positive results of NF training in children with ASD compared to a waiting list control group. Treatment consisted of 40 sessions of neurofeedback and included inhibition of theta activity (4–7 Hz) and rewarding low beta activity (12–15 Hz) over the right hemisphere. It was hypothesized that this induced change in EEG-power would enhance activation of the ACC, which has been found to be under activated in ASD individuals [23]. Consistent with this hypothesis, NF training revealed a linear decrease in theta power and an increase in low beta power over 40 sessions. In the treatment group, there was significant improvement on tasks of executive functioning in the treatment group for attention control, cognitive flexibility, and planning. Measures of social behavior revealed significant improvements in general and non-verbal communication in the treatment but not the control group. Furthermore, parents of children in the treatment group reported more improvement in levels of social interaction, communication, and typical behavior. A follow-up after 12 months revealed maintenance of the described outcomes on both executive functioning and social behavior, suggesting that NF treatment can have long-term effects.

All in all, anecdotal reports, as well as clinical and controlled scientific studies suggest that NF approaches can lead to symptom improvement [140]. Additional randomized and controlled studies are needed to establish "best practices" for NF and determine the optimal set of protocols. Recently, Coben and Meyers [141] compared the results of two published controlled NF studies examining whether a symptom based approach or an assessment/connectivity guided based approach was more effective. Both methods demonstrated significant improvement in symptoms of autism, but connectivity-guided neurofeedback showed a greater reduction on various subscales of the ATEC. Overall, children with autism who successfully reduce delta and theta power through NF therapy have shown improved cognitive flexibility, enhanced social and communicative skills, executive set-shifting functions, and a general decrease of theta power, all of which were maintained long after post-treatment.

Consequences of the hypothesis and discussion

It is hypothesized that operant conditioning methodology, such as NF, produces its behavioral and electrophysiological effects by gaining access to and control over regulatory mechanisms that increase or decrease synchronous or desynchronous activity in brain networks. This assumes that the cortex works in terms of resonant loops and that such functional resonances operate spontaneously or are driven by cellular pacemakers. Oscillations in the network project strong afferent volleys to cortical targets, which could result in a cascade of motor alterations enhanced by long-term potentiation [142]. Furthermore, these changes are stabilized and consolidated over time affecting function beyond the neurofeedback context. Thus, NF is believed to influence, among other things, thalamic pacemakers and consequently thalamocortical resonances. Long-term consequences of this change in activity could induce changes in the patterns of connectivity between different brain regions and ultimately generalized and noticeable improvements in behavior. Primarily a disorder of connectivity, autism is a very suitable target for such treatment.

In a recent review of the literature, Coben et al. [137] argued that while further research is necessary, the variety of studies using neurofeedback in autism support a Level 2 determination ("Possibly efficacious") for the application of neurofeedback for autistic disorders. Nonetheless, it must be acknowledged that a number of limitations characterize many of the NF studies in the field. Given the heterogeneity of ASD, the use of single case studies and small group sizes reduces statistical power. Group studies provide stronger support but there is a pressing need for proper use of random assignment, appropriate control groups, and more blinded protocols to control for placebo effects. Replication by multiple independent laboratories is crucial to establish efficacy, as is the correlation between behavioral changes and functional/structural changes in the brain. Equally important is the need to resolve the discrepancies in outcome measures used, as well as address EEG spatial limitations, perhaps through the use of and comparison with magnetoencephalography- and fMRI-based NF. There is also the clear necessity of delineating common ASD comorbidities, namely ADHD and OCD. Finally, it is important to extend the reach of NF treatments to address the multiple core symptoms of autism – not just social dysfunctions, but also emotional regulation, language problems, and repetitive behaviors. While discrepancies in methodology and outcome measures make comparison between studies difficult, ameliorating these differences will lead to a stronger understanding of NF's efficacy and potential. Our hope is that a continued commitment to overcoming these experimental limitations and challenges will ultimately help establish neurofeedback as a beneficial treatment for children and parents dealing with this difficult disorder.

Conflict of interest statement

The authors have nothing to declare.

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